Reviews of Environmental Contamination and Toxicology

Volume 179

Springer

Reviews of Environmental Contamination and Toxicology

VOLUME 179

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Reviews of Environmental Contamination and Toxicology

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VOLUME 179

Springer

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Springer-Verlag
New York: 175 Fifth Avenue, New York, NY 10010, USA
Heidelberg: Postfach 10 52 80, 69042 Heidelberg, Germany

Library of Congress Catalog Card Number 62-18595.
Printed in the United States of America.

ISSN 0179-5953

Printed on acid-free paper.

Printed in the United States of America.

ISBN 0-387-00620-6 SPIN 10916254

www.springer-ny.com

Springer-Verlag New York Berlin Heidelberg
A member of BertelsmannSpringer Science+Business Media GmbH

Foreword

International concern in scientific, industrial, and governmental communities over traces of xenobiotics in foods and in both abiotic and biotic environments has justified the present triumvirate of specialized publications in this field: comprehensive reviews, rapidly published research papers and progress reports, and archival documentations. These three international publications are integrated and scheduled to provide the coherency essential for nonduplicative and current progress in a field as dynamic and complex as environmental contamination and toxicology. This series is reserved exclusively for the diversified literature on "toxic" chemicals in our food, our feeds, our homes, recreational and working surroundings, our domestic animals, our wildlife and ourselves. Tremendous efforts worldwide have been mobilized to evaluate the nature, presence, magnitude, fate, and toxicology of the chemicals loosed upon the earth. Among the sequelae of this broad new emphasis is an undeniable need for an articulated set of authoritative publications, where one can find the latest important world literature produced by these emerging areas of science together with documentation of pertinent ancillary legislation.

Research directors and legislative or administrative advisers do not have the time to scan the escalating number of technical publications that may contain articles important to current responsibility. Rather, these individuals need the background provided by detailed reviews and the assurance that the latest information is made available to them, all with minimal literature searching. Similarly, the scientist assigned or attracted to a new problem is required to glean all literature pertinent to the task, to publish new developments or important new experimental details quickly, to inform others of findings that might alter their own efforts, and eventually to publish all his/her supporting data and conclusions for archival purposes.

In the fields of environmental contamination and toxicology, the sum of these concerns and responsibilities is decisively addressed by the uniform, encompassing, and timely publication format of the Springer-Verlag (Heidelberg and New York) triumvirate:

Reviews of Environmental Contamination and Toxicology [Vol. 1 through 97 (1962–1986) as Residue Reviews] for detailed review articles concerned with any aspects of chemical contaminants, including pesticides, in the total environment with toxicological considerations and consequences.

Bulletin of Environmental Contamination and Toxicology (Vol. 1 in 1966) for rapid publication of short reports of significant advances and discoveries in the fields of air, soil, water, and food contamination and pollution as well as

methodology and other disciplines concerned with the introduction, presence, and effects of toxicants in the total environment.

Archives of Environmental Contamination and Toxicology (Vol.1 in 1973) for important complete articles emphasizing and describing original experimental or theoretical research work pertaining to the scientific aspects of chemical contaminants in the environment.

Manuscripts for *Reviews* and the *Archives* are in identical formats and are peer reviewed by scientists in the field for adequacy and value; manuscripts for the *Bulletin* are also reviewed, but are published by photo-offset from camera-ready copy to provide the latest results with minimum delay. The individual editors of these three publications comprise the joint Coordinating Board of Editors with referral within the Board of manuscripts submitted to one publication but deemed by major emphasis or length more suitable for one of the others.

Coordinating Board of Editors

Preface

Thanks to our news media, today's lay person may be familiar with such environmental topics as ozone depletion, global warming, greenhouse effect, nuclear and toxic waste disposal, massive marine oil spills, acid rain resulting from atmospheric SO_2 and NO_x, contamination of the marine commons, deforestation, radioactive leaks from nuclear power generators, free chlorine and CFC (chlorofluorocarbon) effects on the ozone layer, mad cow disease, pesticide residues in foods, green chemistry or green technology, volatile organic compounds (VOCs), hormone- or endocrine-disrupting chemicals, declining sperm counts, and immune system suppression by pesticides, just to cite a few. Some of the more current, and perhaps less familiar, additions include *xenobiotic transport, solute transport, Tiers 1 and 2, USEPA to cabinet status, and zero-discharge.* These are only the most prevalent topics of national interest. In more localized settings, residents are faced with leaking underground fuel tanks, movement of nitrates and industrial solvents into groundwater, air pollution and "stay-indoors" alerts in our major cities, radon seepage into homes, poor indoor air quality, chemical spills from overturned railroad tank cars, suspected health effects from living near high-voltage transmission lines, and food contamination by "flesh-eating" bacteria and other fungal or bacterial toxins.

It should then come as no surprise that the '90s generation is the first of mankind to have become afflicted with *chemophobia*, the pervasive and acute fear of chemicals.

There is abundant evidence, however, that virtually all organic chemicals are degraded or dissipated in our not-so-fragile environment, despite efforts by environmental ethicists and the media to persuade us otherwise. However, for most scientists involved in environmental contaminant reduction, there is indeed room for improvement in all spheres.

Environmentalism is the newest global political force, resulting in the emergence of multi-national consortia to control pollution and the evolution of the environmental ethic. Will the new politics of the 21st century be a consortium of technologists and environmentalists or a progressive confrontation? These matters are of genuine concern to governmental agencies and legislative bodies around the world, for many serious chemical incidents have resulted from accidents and improper use.

For those who make the decisions about how our planet is managed, there is an ongoing need for continual surveillance and intelligent controls to avoid endangering the environment, the public health, and wildlife. Ensuring safety-

in-use of the many chemicals involved in our highly industrialized culture is a dynamic challenge, for the old, established materials are continually being displaced by newly developed molecules more acceptable to federal and state regulatory agencies, public health officials, and environmentalists.

Adequate safety-in-use evaluations of all chemicals persistent in our air, foodstuffs, and drinking water are not simple matters, and they incorporate the judgments of many individuals highly trained in a variety of complex biological, chemical, food technological, medical, pharmacological, and toxicological disciplines.

Reviews of Environmental Contamination and Toxicology continues to serve as an integrating factor both in focusing attention on those matters requiring further study and in collating for variously trained readers current knowledge in specific important areas involved with chemical contaminants in the total environment. Previous volumes of *Reviews* illustrate these objectives.

Because manuscripts are published in the order in which they are received in final form, it may seem that some important aspects of analytical chemistry, bioaccumulation, biochemistry, human and animal medicine, legislation, pharmacology, physiology, regulation, and toxicology have been neglected at times. However, these apparent omissions are recognized, and pertinent manuscripts are in preparation. The field is so very large and the interests in it are so varied that the Editor and the Editorial Board earnestly solicit authors and suggestions of underrepresented topics to make this international book series yet more useful and worthwhile.

Reviews of Environmental Contamination and Toxicology attempts to provide concise, critical reviews of timely advances, philosophy, and significant areas of accomplished or needed endeavor in the total field of xenobiotics in any segment of the environment, as well as toxicological implications. These reviews can be either general or specific, but properly they may lie in the domains of analytical chemistry and its methodology, biochemistry, human and animal medicine, legislation, pharmacology, physiology, regulation, and toxicology. Certain affairs in food technology concerned specifically with pesticide and other food-additive problems are also appropriate subjects.

Justification for the preparation of any review for this book series is that it deals with some aspect of the many real problems arising from the presence of any foreign chemical in our surroundings. Thus, manuscripts may encompass case studies from any country. Added plant or animal pest-control chemicals or their metabolites that may persist into food and animal feeds are within this scope. Food additives (substances deliberately added to foods for flavor, odor, appearance, and preservation, as well as those inadvertently added during manufacture, packing, distribution, and storage) are also considered suitable review material. Additionally, chemical contamination in any manner of air, water, soil, or plant or animal life is within these objectives and their purview.

Normally, manuscripts are contributed by invitation, but suggested topics are welcome. Preliminary communication with the Editor is recommended before volunteered review manuscripts are submitted.

Tucson, Arizona G.W.W.

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Physical, Chemical, and Biological Changes in the Gulf of Gdańsk Ecosystem (Southern Baltic Sea)

Agata Kot-Wasik, Barbara Żukowska, Dagmara Dąbrowska, Jolanta Dębska, Józef Pacyna, and Jacek Namieśnik

Contents

I. Introduction

The Baltic Sea is an almost landlocked subsidiary sea to the Atlantic Ocean that is almost totally surrounded by land and therefore is more endangered by pollu-

Communicated by George W. Ware.

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tion than other marine areas. Only narrow straits connect it to the North Sea, and the exchange of water between the two water bodies is therefore restricted. The residence time of water in the central Baltic is estimated as 25–30 years. Numerous rivers drain an area in Central and Northern Europe of more than 1.7 million km^2, transporting approximately 480 km^3 freshwater annually that increasingly dilutes the saline seawater of the Baltic to the east and north. The salt content decreases from about 30 g L^{-1} in the Kattegat deep to 2 g L^{-1} in the innermost areas of the Gulfs of Bothnia and Finland. The catchment area covers 17% of Europe. Total area is about 415,000 km^2 and a volume of water of 21,700 km^3, which means that activities within a land area 4.5 times as large as the area of the sea, and comprising parts of 14 countries, affect the environment of the Baltic. Proceeding from the northern end, it includes Bothnian Bay and the Bothnian Sea. At the southern end of the Bothnian Sea, the island of Aland divides the Aland Sea from the Archipelago Sea. The Gulf of Finland is the eastern arm of the Baltic Sea. The central portion of the Sea, known as the Baltic Proper, includes the Eastern and Western Gotland Seas. To the east and south are the Gulf of Riga and the Gulf of Gdańsk. Moving to the west are the Bornholm and Arkona basins, followed by the Sound, the Belt Sea, and the Kattegat. The Baltic Sea, including the Kattegat, is one of the world's largest areas of brackish water (Rheinheimer 1998; Szefer 2002).

Nine countries share the Baltic Sea coastline: Sweden and Finland to the north, Russia, Estonia, Latvia, and Lithuania to the east, followed by Poland in the south, and Germany and Denmark in the west. About 16 million people live on the coast, and around 80 million in the entire catchment area of the Baltic Sea. The catchment area includes part of Belarus, the Czech Republic, Norway, the Slovak Republic and Ukraine, as some of the rivers find their sources here. About 140 million people live in the nine countries surrounding the Baltic Sea (BOING 2001).

Regular measurements carried out within the framework of the Helsinki Commission Baltic Monitoring Programme (HELCOM 1997) point to eutrophication as the major ecological problem of the Baltic (Łysiak-Pastuszak et al. 2000).

II. Polish Coastal Zone of the Baltic Sea

The Baltic Sea forms 843 km of the Polish borderline, or 15% of the total length of the country's border (i.e., 102 km of the Vistula Lagoon, 241 km of the Pomeranian Bay, 76 km of the Hel Peninsula, and 424 km of the remaining part of the coast). Some 99.7% of the country is situated within the Baltic Sea drainage area, which covers 311,900 km^2 (Fig. 1).

Poland is one of the major countries that has considerably influenced the condition of the Baltic Sea. The population of Poland constitutes 50% of the whole population living in the basin of Baltic Sea. Most of Poland's territory is located within two catchment areas of the two biggest rivers: the Vistula River (54% of country area) and the Odra River (33.9%).

The most polluted areas of the Polish coastal waters are the Gulf of Gdańsk

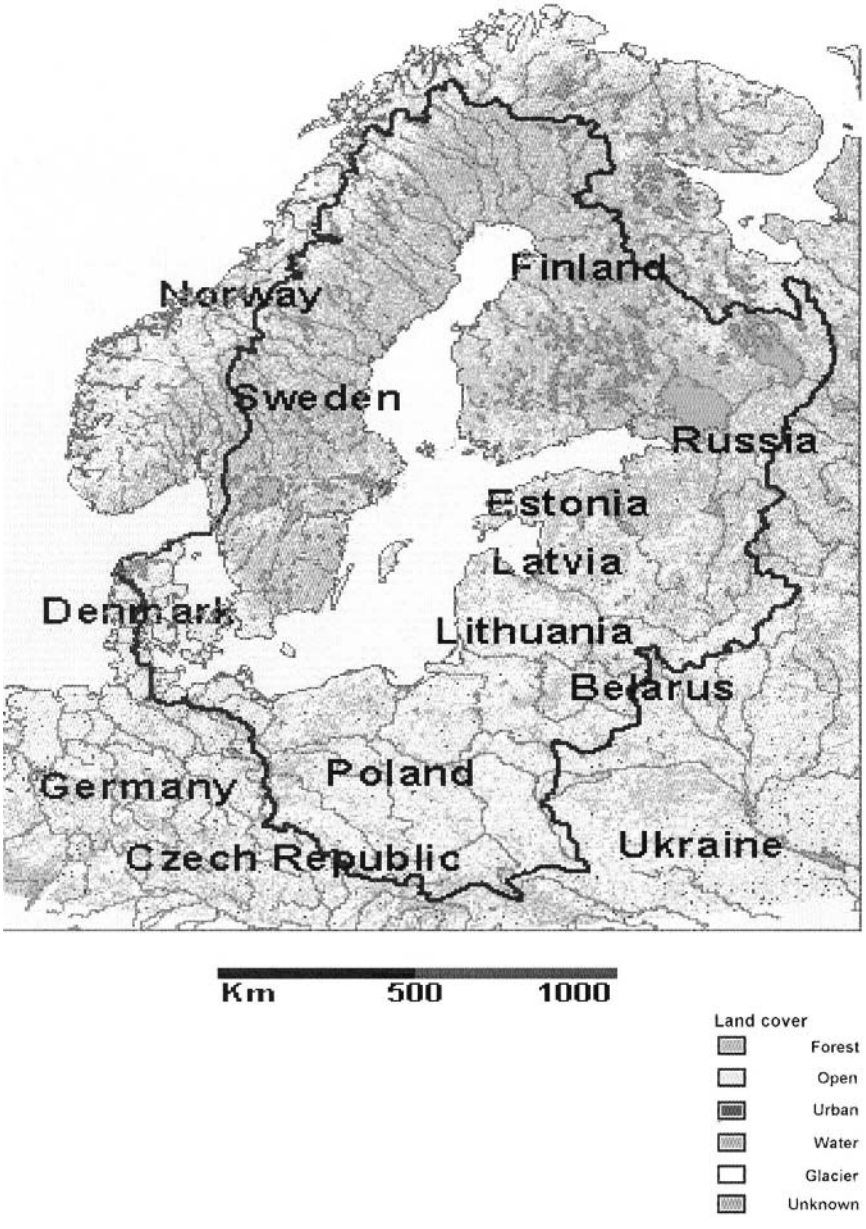

Fig. 1. Drainage area of the Baltic Sea. (From Ahlenius 2000.)

and the Pomeranian Bay, both of which absorb significant pollution loads through river outflows. Intensive primary production has been observed in these areas. Along the more open Polish coast, the problems are similar to those in the open Baltic Sea. The symptoms of eutrophication in the Baltic Sea were first noticed 30–40 years ago, first in the basin adjacent to land sources of emission of nitrogen and phosphorus compounds. Changes occurring within the first trophic level, i.e., increase of phytoplankton biomass and abundance, exert a negative influence on the entire ecosystem of the Baltic Sea, including species composition and population structure at various levels of the food web (Cederwall and Elmgren 1990; Rydberg et al 1990; Smayda 1997), oxygen conditions, and the biogeochemical cycle of nutrients (HELCOM 1993, 1996; Łysiak-Pastuszak et al. 2000). In the late 1980s, hydrogen sulfide was detected in the Gulf of Gdańsk in high concentrations. The decrease in fish catches along the entire Polish coast during the past decade has been attributed to changes in living conditions for fish, but overexploitation of certain fish stocks may have played an important role in these changes as well (Nowacki and Jarosz 1998).

A. Gulf of Gdańsk

The Gulf of Gdańsk (Fig. 2) straddles the border of Poland and the Kaliningrad Oblast (Russia) along the southern coast of the Baltic. Excluding the Vistula Lagoon, the total estimated surface area of the Gulf of Gdańsk is 4,296 km^2; land area is 304,510 km^2 and the coastline 491 km, with the volume of the Gulf estimated at 236 km^3 (Witek et al. 2000).

The Gulf of Gdańsk consists of several morphological subunits: the Vistula Lagoon, an almost completely landlocked and anthropogenically stressed area, the semienclosed Bay of Puck, and the mouth of the Vistula. Along the southern coast of the Gulf of Gdańsk spreads the Gdańsk–Sopot–Gdynia agglomeration area, with a total population reaching 750,000 inhabitants.

Maximum depth in the Gdańsk Deep is 118 m. The Gulf of Gdańsk is a rather shallow water basin with a sandy bottom, separated from the Baltic Proper by the Hel Peninsula, which limits the exchange of water. A comparison of selected properties of the Gulf of Gdańsk and the Baltic Sea is presented in Table 1.

B. Ecosystem Changes in Brief

Annual freshwater discharge into Gulf of Gdańsk is 34.5 km^3, of which the Vistula River contributes approximately 30%, which is approximately 7% of the total input of freshwater (Glasby and Szefer 1998). About 5%–10% of the time the Vistula River water discharged into the Baltic flows westward, resulting in dispersion of pollutants onto the beaches of the Gulf of Gdańsk and Puck Bay (Szefer et al. 1996). More than 90% of the pollutants observed in the Gulf of Gdańsk is carried by the Vistula River (Table 2).

Throughout the past 30 years, an increase in eutrophication evidently resulting from the massive load of nutrients carried by the Vistula River has been

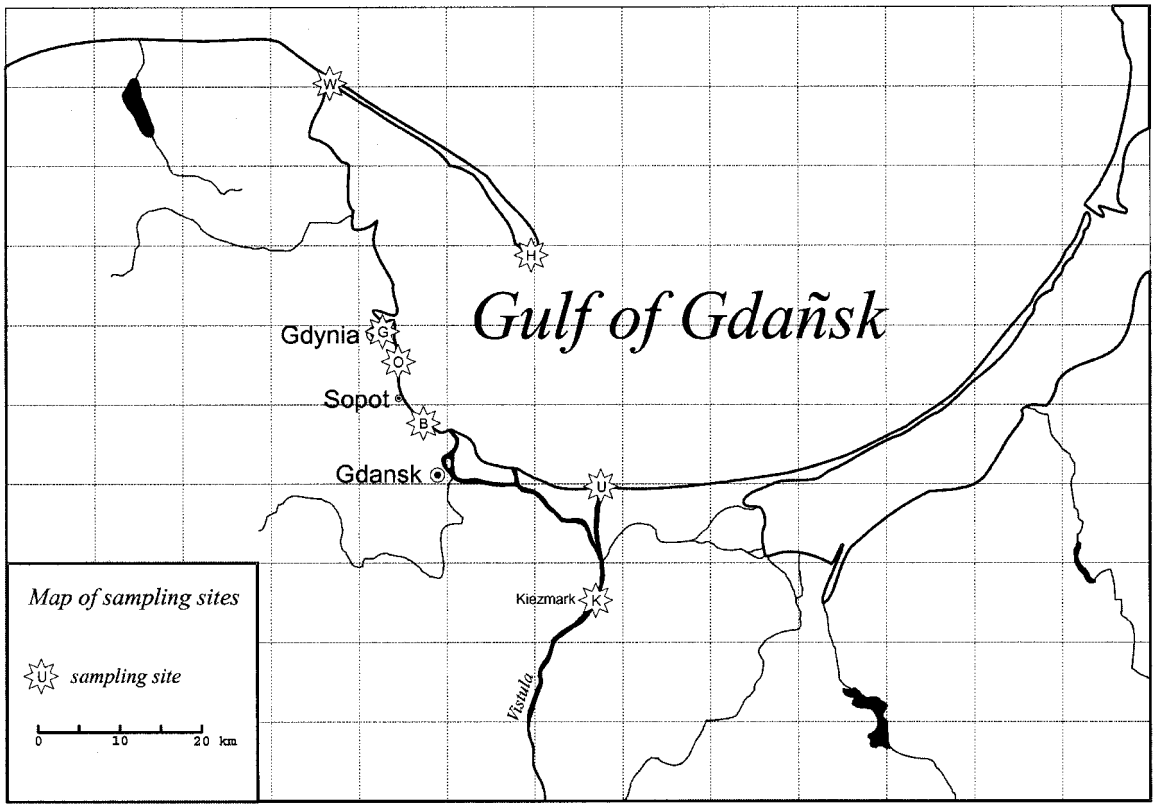

Fig. 2. The Gulf of Gdańsk (18°22′–20°00′ E, 54°18′–54°50′ N).

Table 1. Comparison of selected characteristics of the Gulf of Gdańsk and the Baltic Sea.

	Gulf of Gdańsk	Baltic Sea
Volume		
km^3	82.8	21,714
%	0.38	100
Area		
km^2	291	415,000
%	0.0007	100
Catchment area		
km^2	197,165	1,649,550
%	11.9	100
Population		
mln M	26	80
%	32.5	100
M/m^3	314	3.6
Arable lands		
km^2	93,719	396,501
%	23.6	100
km^2/km^3	314	33.7
River water		
km^3/year	32.5	443.6
Water change %/year	0.39	2.2

Source: Blazejowski and Schuller (1994).

Table 2. Comparison of yearly load of pollutants entering the Gulf of Gdańsk from the Gdańsk area and Vistula River.

Sources of pollutants	Outflow size, 10^3 m^3/yr (%)	BOD, tO_2/yr (%)	COD, tO_2/yr (%)	Nog, t/yr (%)	Pog, t/yr (%)	Suspended materials, t/yr (%)
The Gdańsk area	236,996 (0.58)	1,691.5 (1.19)	6,237.6 (1.66)	1,240.9 (1.17)	85.8 (1.17)	6,144.2 (0.96)
Vistula	40,873,284 (99.42)	140,178.8 (98.81)	369,784 (98.34)	113,203 (98.83)	7,249 (98.83)	632,906 (99.04)
Total	41,110,280 (100.0)	141,870.3 (100.0)	376,021.6 (100.0)	376,021.6 (100.0)	7,334.8 (100.0)	639,050.7 (100.0)

BOD: biological oxygen demand; COD: chemical oxygen demand; Nog: total NITROGEN; Pog: total PHOSPHORUS.

Source: Kopeć et al. (2001).

observed. Sediment studies have revealed that the content of heavy metals in the sediments has increased. Oxygen deficiency and hydrogen sulfide presence are features commonly encountered in the bottom layers. Meanwhile, the ecosystem structure of the Gulf of Gdańsk has undergone tremendous changes: the species pattern has changed significantly, many species have disappeared, and some species have become dominant. The most important changes to the ecosystem caused by anthropogenic pollution are presented in Table 3.

III. Climate in the Coastal Zone

The climate features of the narrow coastal belt are under the influence of the Baltic Sea and the Atlantic Ocean, and relocating low pressures, especially in late fall and winter as well as periodically occurring high pressures, occur partic-

Table 3. The most important changes in the ecosystem caused by anthropogenic pollution.

Year	Change
End of 1960s	Significant increase in nutrient concentration.
Beginning of 1970s	Increases in the amounts of suspended matter and considerable drop in water transparency.
Mid-1970s	Extinction of *Fucus vesiculosus* and *Furcellaria lumbricalis* in the Puck Lagoon, an inner part of the bay.
Since 1975	Changes in the fish populations; e.g., roach, cod, pike, perch, and garfish are disappearing; the sticklebacks *Gasterosteus aculeatus* and *Pungitus pungitus* have become more abundant.
1980s	Shrinking of underwater grass meadows and fish spawning areas; breakup of coastal fishery.
From 1985	Abundance of protozoa; intensive blooms of brown algae (*Ectocarpales*) and phytoplankton.
1993	Area of algal blooms in the Gulf of Gdańsk increased by 7% compared to 1992.
1994	First recorded bloom of toxic blue-green alga *Nodularia spumigena*.
1995	Appearance of a gill neoplasia in the bivalve *Macoma baltica*.
1991–1995	Almost no watering places were available in the coastal zone of the Gulf of Gdańsk (beaches along Tricity coastline were closed).
From 1996	The number of watering places has been changing continuously. In 1998, in all sampling points situated along the seashore of the Gulf of Gdańsk, 70% of water belonged to class I and II (absence of bacteria and petroleum pollutants permits classifying that water as "admissible for recreation"). For more than 26 km of seashore in the Gdańsk area, water belongs to class I or II.
1999	Currently, no untreated wastes are discharged directly into the Gulf of Gdańsk (Gdańsk City Hall 1999).

Sources: Celej et al. (2001); Mojski (2000).

ularly in midspring or fall (Agricultural University of Szczecin 2000; Institute of Meteorology and Water Management 1999; Koźmiński et al. 2000; Orłowicz 1996; Romer 1949; Woś 1999).

A. Water Temperature

Seasonal air temperature distribution in the coastal zone causes winters to be usually quite mild and warm, especially in the western part, and a chilly spring occurs in the northeast. Annual temperature fluctuations range from 17.1 °C in Ustka up to 19.4° in Elbląg, increasing to the east along the coast and to inland. Annual average water temperatures in the past 10 years along the Polish coastal zone are presented in Fig. 3.

B. Precipitation

A shifting coastline in the sea is conducive to the inflow of rain-carrying winds from the west or northwest that leads to the increase of total precipitation. Total average annual precipitation varies from roughly 320 mm around Rozewie and Gdynia to 720 mm in Elbląg, with the exception of precipitation in 1993, when in Elbląg it was as low as 100 (Fig. 4). The highest precipitation usually occurs in July and August and the lowest in February and March. In the coastal zone, the autumns are superior to the springs (Omstedt et al. 1997).

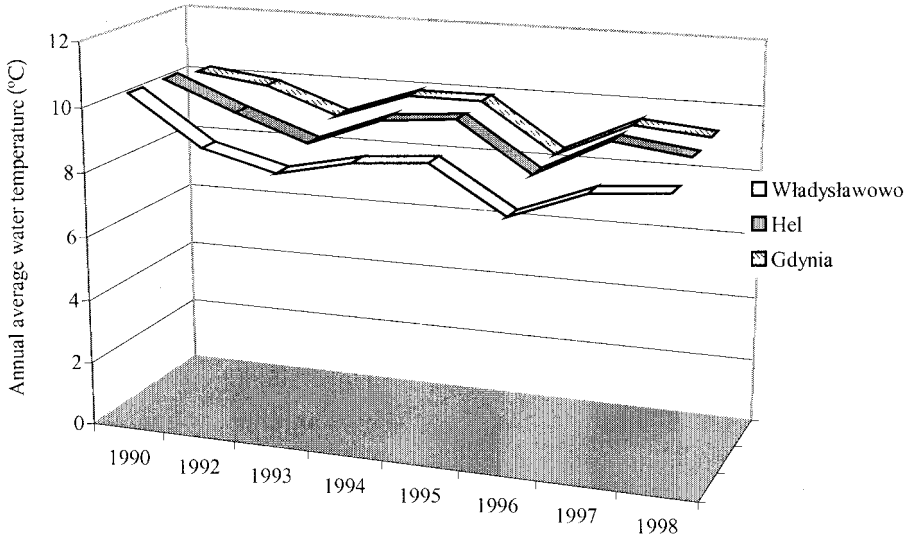

Fig. 3. Annual average water temperature (°C).

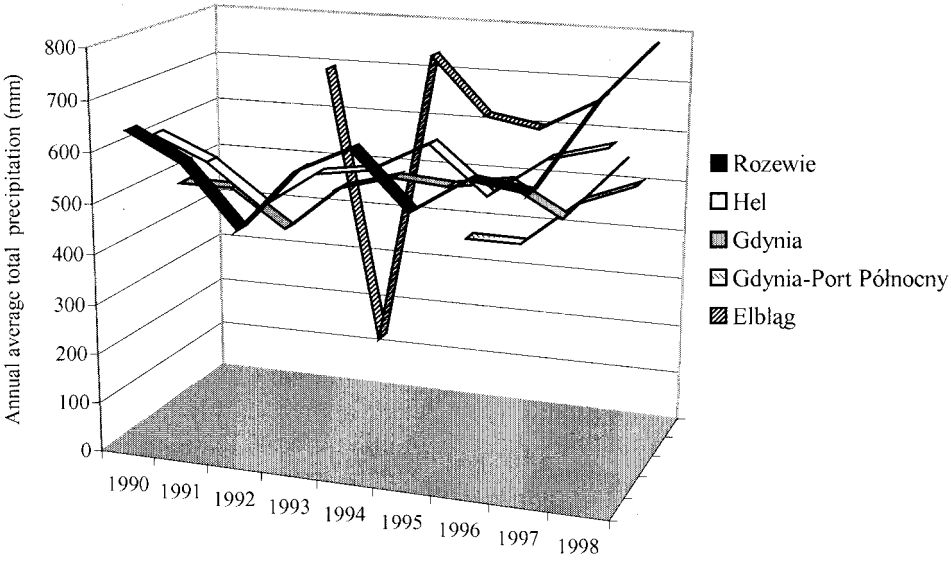

Fig. 4. Annual average total precipitation (mm).

C. Sea Level Changes

The most important changes in the Baltic Sea level observed on the Polish coast are as follows (Rotnicki and Borzyszkowska 1999).

- The sea level rise over the years 1951–1990 was very distinct; it was the least in the west (Świnoujście, 2.19 mm yr^{-1}), more marked in the middle coast (Kołobrzeg, 2.19 mm yr^{-1}; Ustka, 2.56 mm yr^{-1}), and most in the east (Gdańsk, 4.02 mm yr^{-1}).
- When the period is divided into two 20-yr intervals (1951–1970 and 1971–1990), the sea level rise is clearly shown to have increased over the past 20 yr (Świnoujście, 4.38 mm yr^{-1}; Kołobrzeg, 5.84 mm yr^{-1}; Ustka, 4.38 mm yr^{-1}; Gdańsk, 7.67 mm yr^{-1}). This increase is not a matter of the past half-century.
- Comparison of the two-decade periods 1951–1970 and 1971–1990 shows a decrease in the frequency of low water levels by 26%–37%, no change in the frequency of middle levels, and a substantial increase in the frequency of high water levels (from 70% in Świnoujście to 154% in Gdańsk). Among high water levels, there has been a steep increase in frequency of storm surges; in the latter 20-yr period it increased with respect to the first by 79% in Świnoujście, 130% in Kołobrzeg, 163% in Ustka, and by as much as 687% in Gdańsk. Hence, we can talk of marked changes in the high water and storm surge regimen of the southern Baltic. Annual mean water levels along the Polish coast are presented in Fig. 5.

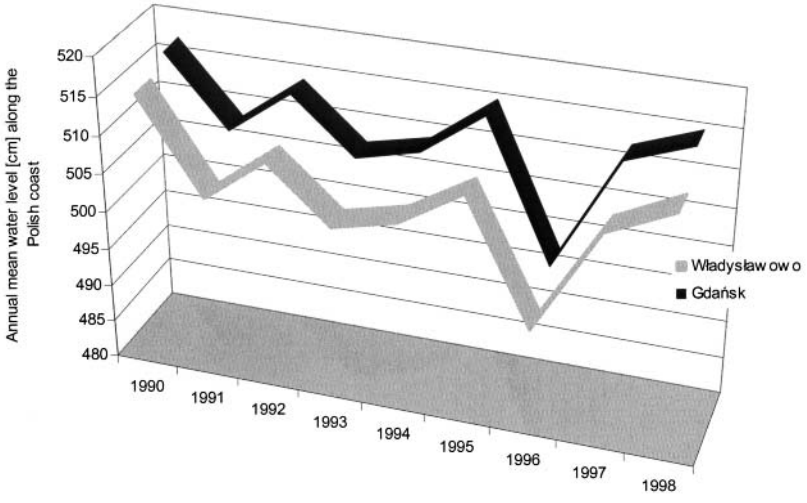

Fig. 5. Annual mean water level (cm) along the Polish coast.

D. Salinity

Surface salinity in the southern Baltic varies from 6% in winter up to 8% in summer. The surface water salinity in the Gulf of Gdańsk varies between 7.3% and 8.4% but is somewhat lower near the mouth of the Vistula River (5.5–6.2%). Salinity is strongly influenced by the outflow from the Vistula River, and salinity (a hydrological front) occurs about 10 km from the river mouth. The lowest salinity near the mouth of Vistula is about 4.5% in spring and summer (Nowacki and Jarosz 1998). The outflow is transported mainly to the east, northeast, and north (Glasby and Szefer 1998; Szefer et al. 1996).

Detailed study of the Baltic Sea in 1998 showed that thermal conditions and salinity did not essentially differ from those in the previous years. Neither a significant drop of temperature of the surface layer in winter nor a considerable rise in summer was observed. The bottom layer of the sea was supplied with waters from the North Sea, which led to an oxygen deficiency.

E. Oxygen Content

Oxygen content of coastal zone waters varied widely in both seasons. In the warm season, oxygen saturation fell to 22.3%; in the cold season, this value never dropped below 57%. It seems that owing to the large amount of nutrients in the coastal zone the primary production in the vegetative season intensifies, which results in increase of oxygen concentration above 12 $cm^{-3}dm^3$ (Falkowska et al. 1993). Detailed data are presented in Table 4.

In 1994 and 1998, the lowest oxygen content in the bottom layer of deep-water areas were found. In the coastline area, these were in the range of long-

Table 4. Seasonal variations of oxygen concentration (μmol dm^{-3}) and saturation (%) in the individual zones of the Gulf of Gdańsk, 1986–1991.

Zone	Estimator	Warm season	Cold season	1986–1991
Concentration				
Inner Puck Bay (surface–bottom)	$\bar{x}$	6.78	7.91	7.14
	min	3.70	5.87	3.70
	max	10.40	11.07	11.07
Outer Puck Bay (surface–bottom)	$\bar{x}$	7.11	7.92	7.38
	min	2.80	3.49	2.80
	max	11.08	10.04	11.08
Coastal zone (0–20 m)	$\bar{x}$	7.08	7.76	7.31
	min	3.28	4.92	3.28
	max	12.60	11.94	12.60
Euphotic layer	$\bar{x}$	7.25	7.94	7.47
Open part of the Gulf of Gdańsk	min	3.35	3.64	3.35
	max	11.95	11.22	11.95
Aphotic layer	$\bar{x}$	6.41	7.22	6.65
Open part of the Gulf of Gdańsk	min	0.00	1.51	0.00
	max	9.33	8.77	9.33
Saturation				
Inner Puck Bay (surface–bottom)	$\bar{x}$	97.4	90.8	95.5
	min	50.7	58.2	50.7
	max	144.0	118.9	144.0
Outer Puck Bay (surface–bottom)	$\bar{x}$	95.3	95.4	95.3
	min	57.0	42.3	42.3
	max	140.7	124.6	140.7
Coastal zone (0–20 m)	$\bar{x}$	96.2	92.3	94.9
	min	22.3	57.9	22.3
	max	177.2	124.1	177.2
Euphotic layer	$\bar{x}$	97.9	94.9	96.9
Open part of the Gulf of Gdańsk	min	47.9	48.1	47.9
	max	146.4	142.0	146.4
Aphotic layer	$\bar{x}$	88.2	84.3	87.0
Open part of the Gulf of Gdańsk	min	0.0	18.0	0.0
	max	107.7	115.5	115.5

$\bar{x}$: mean value; min: minimum value; max: maximum value.
Source: Falkowska et al. (1993).

term changes. Flood water entering the Gulf of Gdańsk in August 2001 contained less oxygen than the surrounding marine water; hence, a stratification of oxygen content appeared in the water column with the minimum in the surface layer. In February 2002, because of inflow of salty water into the Gdańsk Deep, oxygen content near the bottom increased to 2.4 cm dm^{-3}, being the highest in this season in the decade 1992–2001.

IV. Nutrient Fluctuations

The fastest rates of increase in Baltic Sea productivity were noted in the 1960s and 1970s (HELCOM 1987). In the 1980s, increase of concentrations of assimilable phosphate and nitrogen compounds slowed (Trzosińska and Łysiak-Pastuszak 1996). Despite the fact that by around 1990 the input of nitrogen and phosphorus salts into the Baltic Sea from land-based sources had dropped significantly (HELCOM 1993), the amounts of nitrogen and phosphorus accumulating in the upper layers of the sea were still sufficient to sustain primary productivity at a considerably elevated level (Łysiak-Pastuszak 2000). High concentrations of biogenic compounds resulted in visible symptoms of eutrophication.

A. Nitrate and Phosphate

The highest increase rate in nutrient concentrations, and hence the greatest increment in productivity, was observed in the Baltic Sea during the 1960s and 1970s (Łysiak-Pastuszak et al. 2000; Nehring and Matthäus 1990; Trzosińska 1977, 1990). The 1980s witnessed considerable reduction in nitrogen and phosphorus load discharged into the Baltic Sea from land sources (HELCOM 1993), and this resulted in a delayed decrease in winter concentrations of these nutrients in seawater, particularly noticeable at the beginning of the 1990s (Cyberska et al. 1990–1999; Łysiak-Pastuszak et al. 2000; Trzosińska and Łysiak-Pastuszak 1996). According to HELCOM recommendations (HELCOM 1987, 1990, 1996; SEPA 2000), long-term trends in nutrient concentrations are assessed in the surface (0–24 m) layer of the sea because they yield information about the supply of nutrients in the open sea–pelagic zone. Figure 6 illustrates the results of linear regression analysis of winter concentrations of nutrients in the euphotic zone of the Gdańsk Deep (Łysiak-Pastuszak et al. 2000).

The highest seasonal variability of phosphates was found in the inner part of the Gulf of Gdańsk (Vistula Mouth). As in 1989–1993, the respective seasonal fluctuations of phosphate in the Gulf, the basins most exposed to land-based discharges of phosphorus, were 0.5 and 0.8 mmol m^{-3} (Inspectorate of Environmental Protection 1999). The largest winter accumulation of phosphates in the surface layer is still being recorded. However, the degree of winter accumulation in 1994–1998 was noticeably lower than that in the previous monitoring period (1989–1993). The declining accumulation of phosphate in the surface was recorded for all regions of the Polish part of the Baltic Sea. The regular seasonal development of phosphate and also that of nitrate and silicate in the Gulf was altered in the summer of 1997 because of the flood in the Vistula catchment's area, even though the impact of the flood waters was restricted to bay areas and was not detected either along the Polish central coast or in the open sea. Figure 7 shows the mean seasonal development of nutrient fluctuations in the Polish coastal zone (Łysiak-Pastuszak 2000). Comparison of nitrogen and phosphorus loads entering the Baltic Sea from the Vistula, Oder, and other rivers is presented in Fig. 8.

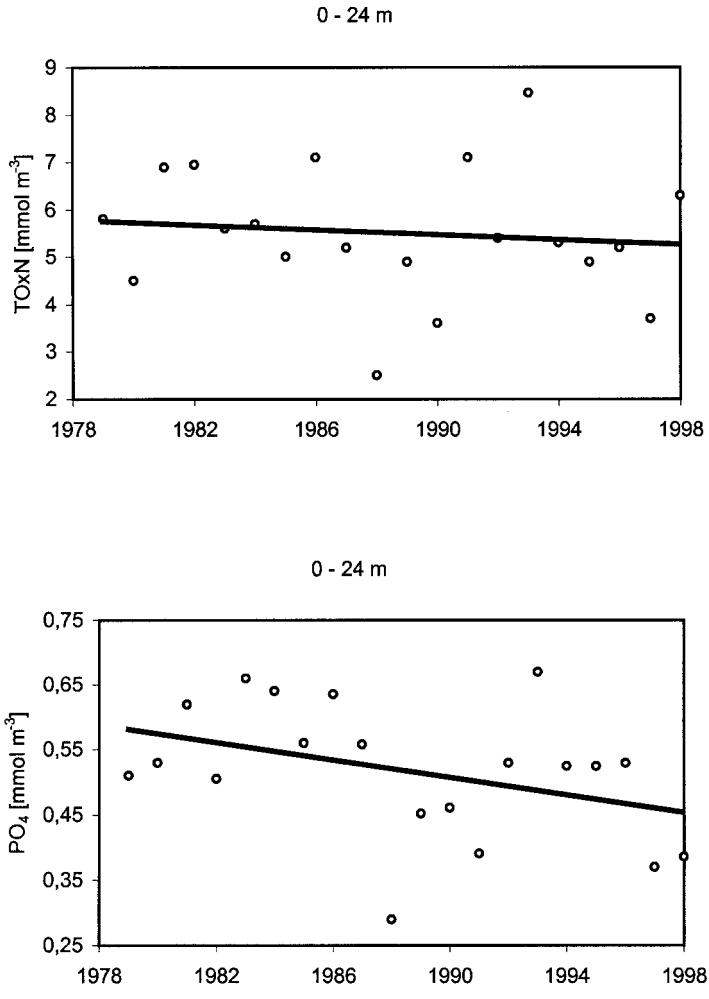

Fig. 6. Variability in nitrate and phosphate concentrations during winter accumulation in the 0–24 m water layer of the Gdańsk Deep (station P1 = BMP L1), 1979–1998.

B. Silicate

Silicates are important in the growth of diatoms. A considerable decrease in winter silicate concentrations in the upper (0–10 m) water layer of the Gdańsk Basin between 1989 and 1993 was recorded. This tendency continued in the subsequent period, but mainly in the offshore part of the Gdańsk Basin and along the central Polish coast (Fig. 9).

Silicate concentrations remained at the same level as between 1989 and 1993, or increased but only slightly, because of the continuous high riverine supply. The mean annual concentrations of silicate decreased in the open-sea area,

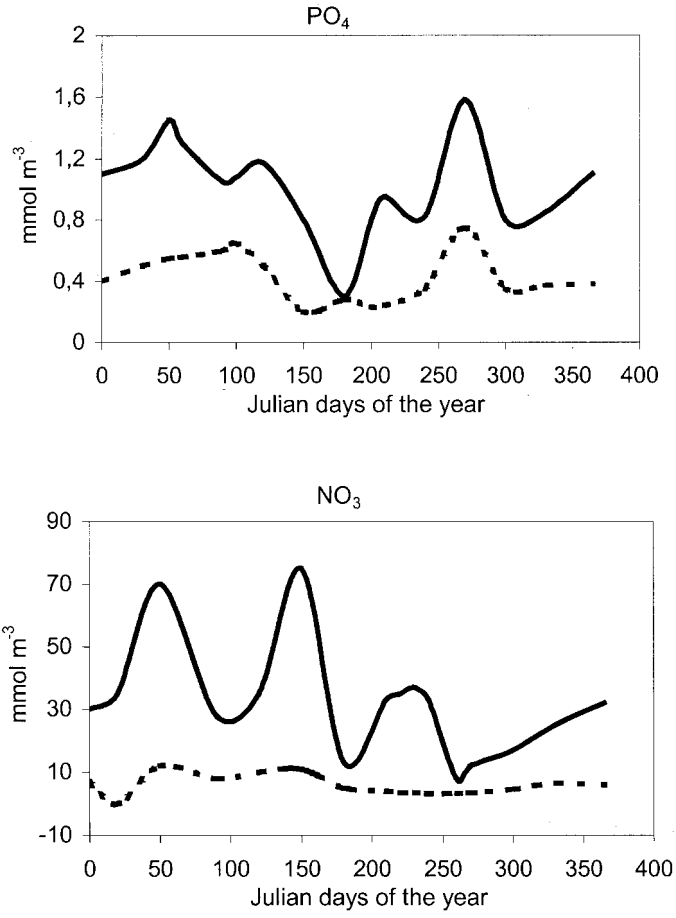

Fig. 7. The mean (1994–1998) seasonal nutrient fluctuations in the coastal zone: inner part of the Gulf of Gdańsk Vistula Mouth (*solid line*, 0–4 m; *dashed line*, 15 m to bottom).

throughout the isohaline layer. An important divergence in the seasonal development of silicate concentrations in 1994–1998, as compared to 1989–1993, was the delayed stabilization of the winter plateau in regions of the Gdańsk Deep (Inspectorate of Environmental Protection 1999).

V. Toxic Pollutants

A. Heavy Metals

Comparison of metals input to the Baltic Sea (t/yr) by natural river and atmospheric inputs, total fluvial and airborne inputs, the sum total for the Baltic Sea, and the respective anthropogenic share is presented in Table 5.

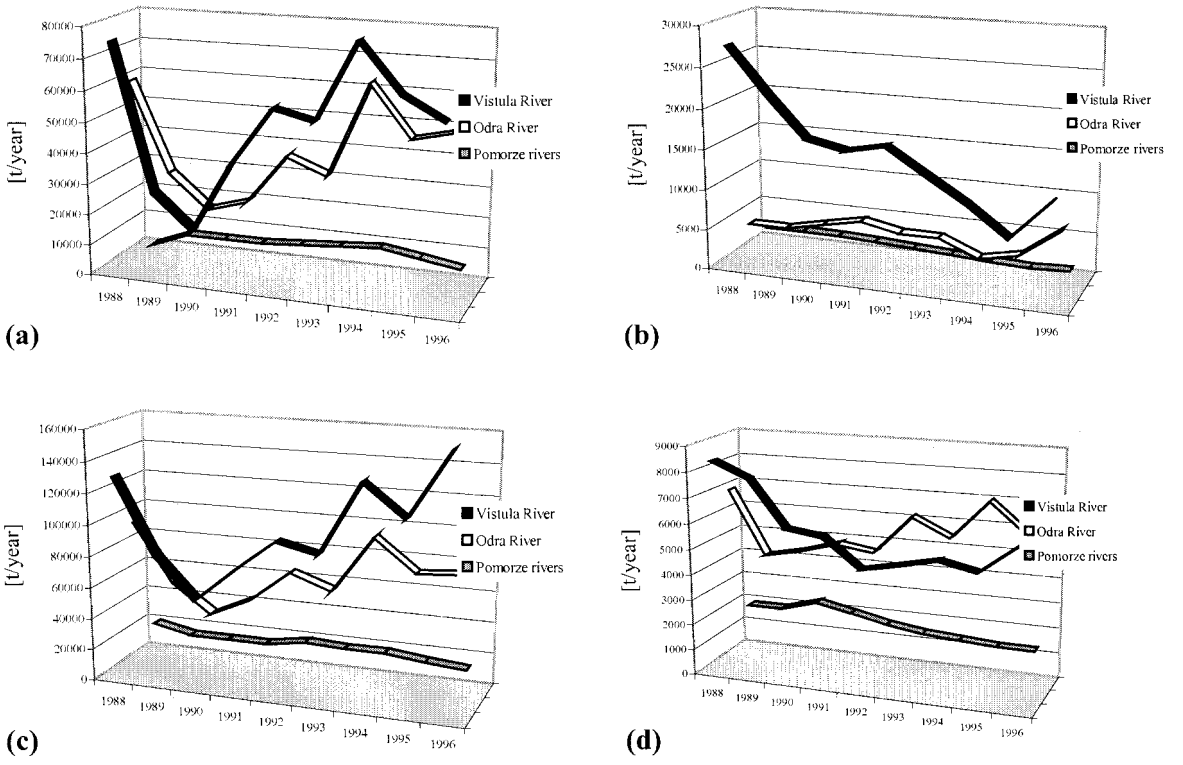

Fig. 8. Comparison of nitrogen and phosphorus load entering the Baltic Sea from the Vistula, Oder, and Pomorze Rivers within 1988–1996: (a) changes in loads of $N\text{-}NO_3$; (b) changes in loads of $N\text{-}NH_4$; (c) changes in loads of Nc; (d) changes in loads of P.

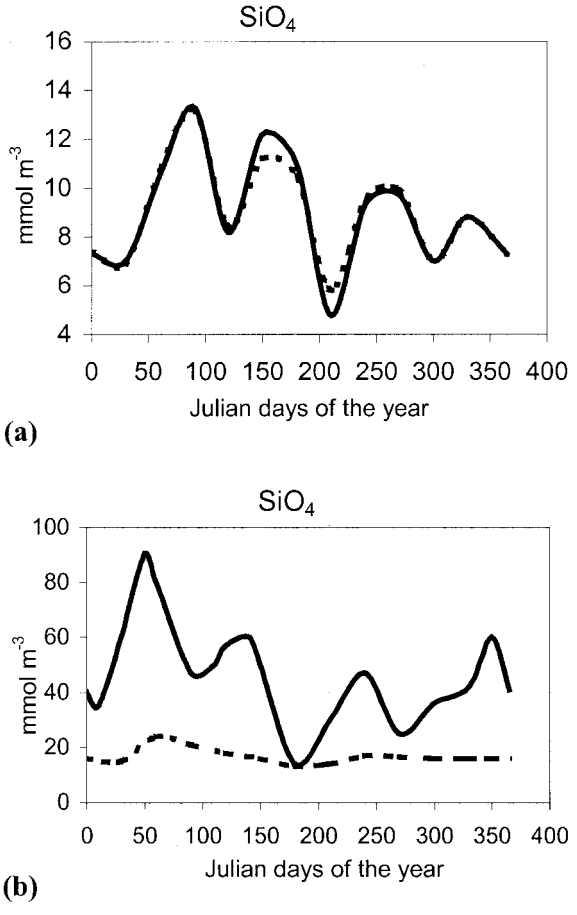

Fig. 9. The mean (1994–1998) seasonal changes of silicate in the coastal zone: (a) central Polish coast; (b) inner part of the Gulf of Gdańsk Vistula Mouth (*solid line*, 0–4 m; *dashed line*, 15 m to bottom). (From Łysiak-Pastuszak 2000.)

The high levels of some metals introduced from the atmosphere result from heavy motor vehicle traffic. The results of measuring copper, lead, cadmium, and zinc in surface and demersal waters of the Gulf of Gdańsk in the 1980s allows for the following conclusions (Bolałek et al. 1988):

- Concentration of dissolved copper varied from 0.55 to 5.4 μg/dm^3, whereas that of suspended copper was from 0.05 to 4.4 μg/dm^3. During summer months concentration of the dissolved form was slightly higher than during winter; the opposite phenomenon being seen in the suspended form.
- Concentration of dissolved lead varied from 0.20 to 2.2 μg/dm^3. Maximum values of the concentration were found in samples collected in February and November, which should be related to an the influx from the atmosphere.

Table 5. Metal inputs to the Baltic Sea (t/yr) from different sources.

Sources	Cd	Cu	Pb	Zn
Natural fluvial	5.1	310	140	1,700
Natural atmospheric	3	130	20	350
Total natural	8.1	440	160	2,050
Fluvial and diffuse	60	1,300	1,500	6,000
Atmospheric	60	1,200	1,300	5,000
Total to Baltic Sea	120	2,500	1,800	11,000
From Vistula River	10.7	73.5	103.6	462
Percent anthropogenic	93	82	91	81

Source: Matschullat (1997).

- Concentration of dissolved cadmium varied from 0.05 to 0.75 μg/dm^3 whereas that of the suspended form was 0.02–1.0 μg/dm^3; the values exceeding 0.25 μg/dm^3 were found during winter months.
- Concentration of dissolved zinc during summer months varied in a wider range, from 3.2 to 17.4 μg/dm^3, than during winter months, from 4.2 to 12.6 μg/dm^3, the opposite phenomenon occurring in suspended matter in the summer season, from 0.8 to 12.8 μg/dm^3, and in winter, from 3.7 to 30 μg/dm^3.

In the past 5 yr, selected heavy metals dissolved in seawater have been under investigation; results for places situated along the Polish seashore of the Gulf of Gdańsk are shown in Fig. 10.

The most recent data proved that the highest concentrations of Zn, Cd, Pb, and Cu were observed in Kiezmark and Orłowo and the lowest in Hel (Biziuk 2001; Biziuk et al. 2001). Generally, concentrations of these metals have slowly decreased in the past 6 yr. As also shown in Fig. 10, the flood observed in July 2001 did not elevate the total concentrations of Zn, Cd, Pb, or Cu.

Changes in loads for heavy metals in southern part of the Baltic Sea within 1996–1998 are shown in Table 6. As seen in Table 6, considerable decrease of loads of selected heavy metals was observed between 1992 and 1998. This finding compares with the average amounts of some selected pollutants accumulated in Baltic fishes in 1995 and 1997, in which lesser amounts of lead and mercury were observed. However, in 1997, an increased level of cadmium was noted in the Baltic sprat.

B. Persistent Organic Compounds

Persistent organic compounds have for decades been widely dispersed in the Baltic marine environment. These stable compounds accumulate in fatty tissues of fish, shellfish, seabirds, and seals, causing sterility, varying degrees of deformation, damage to vital organs, and several other forms of disease in the animals (BOING 2001).

In the Helsinki Convention, PCBs and DDTs are included among the sub-

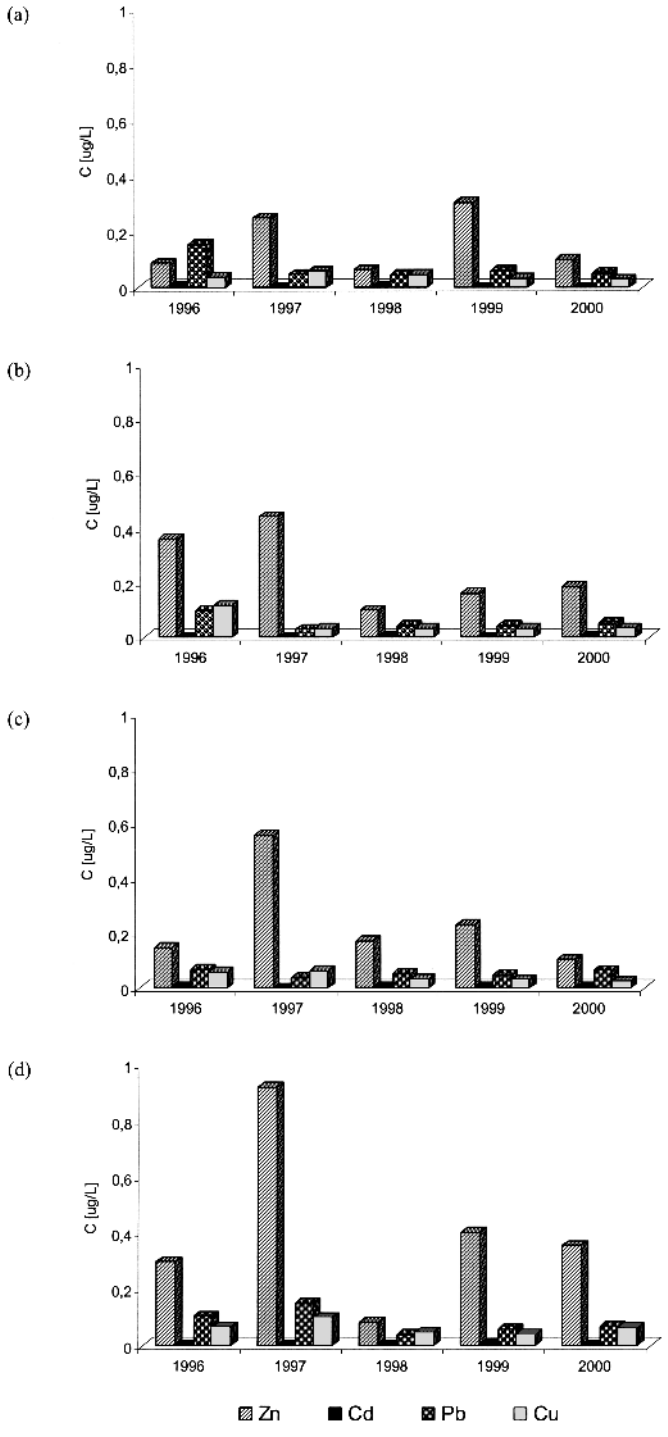

Fig. 10. Annual average concentrations of selected heavy metals [μg/L] in the Gulf of Gdańsk and the Vistula River water in (a) Hel, (b) Władysławowo, (c) Orłowo, and (d) Kiezmark in 1996–2000.

Table 6. Annal loads for selected heavy metals (t/yr).

	1992	1993	1994	1995	1996	1997	1998
Total iron	19,635	12,825	25,026	27,088	20,713	8,131	8,356
Manganese	4,878	4,883	6,438	6,157	4,010	2,380	1,927
Total chromium	—	59	69	52	12	6	28
Zinc	1,740	1,175	1,006	852	511	497	473
Cadmium	47	25	18	9	8	5	4
Copper	276	203	181	135	116	134	116
Lead	298	370	215	127	71	62	37

Source: Landsberg (2001).

stances banned in the Baltic Sea area. Restrictions are imposed on their transport, trade, handling, use, and disposal.

C. PCBs

Polychlorinated biphenyl content in waters in the area of the Gulf of Gdańsk has been determined for a long time (Fig. 11). Generally, very low concentrations of PCBs have been observed. Figure 11 shows that an unusually high concentration of PCBs was noted in 1999. Generally, a decreasing tendency of PCB concentration in seawater of the Gulf of Gdańsk has been noted.

According to data from national monitoring programs, in fish (herring and cod) and the eggs of guillemot (a seabird) in the Baltic Sea area, levels of PCBs have in recent years decreased significantly. However, although the concentrations of PCBs in living organisms in the Baltic Sea are clearly decreasing, they are still unacceptably high. The input of PCBs leaching from land and from sediments will continue in this area, and it has also been demonstrated that airborne PCBs account for a considerable portion of the total PCB load in the Baltic today. Relatively high concentrations of PCBs are still observed in the case of sediments, as shown in Table 7 (Konat and Kowalewska 2001).

Total content of PCBs in recent sediments of the Baltic Sea during 1996–1999 is shown in Fig. 12. Note that total PCB content in sediments in the Gulf of Gdańsk is relatively low in comparison to the content in the Gdańsk Deep.

D. Pesticides

Concentrations of selected pesticides observed in the Gulf of Gdańsk within the past 6 yr are presented in Fig. 13.

Today, the annual average concentration of each from analyzed pesticides is very low, lower than in recent years. Loads for these particular compounds are summarized in Table 8. Some pesticides are also known for their tendency to bioaccumulate; therefore, detailed investigations on their levels in different organisms are required.

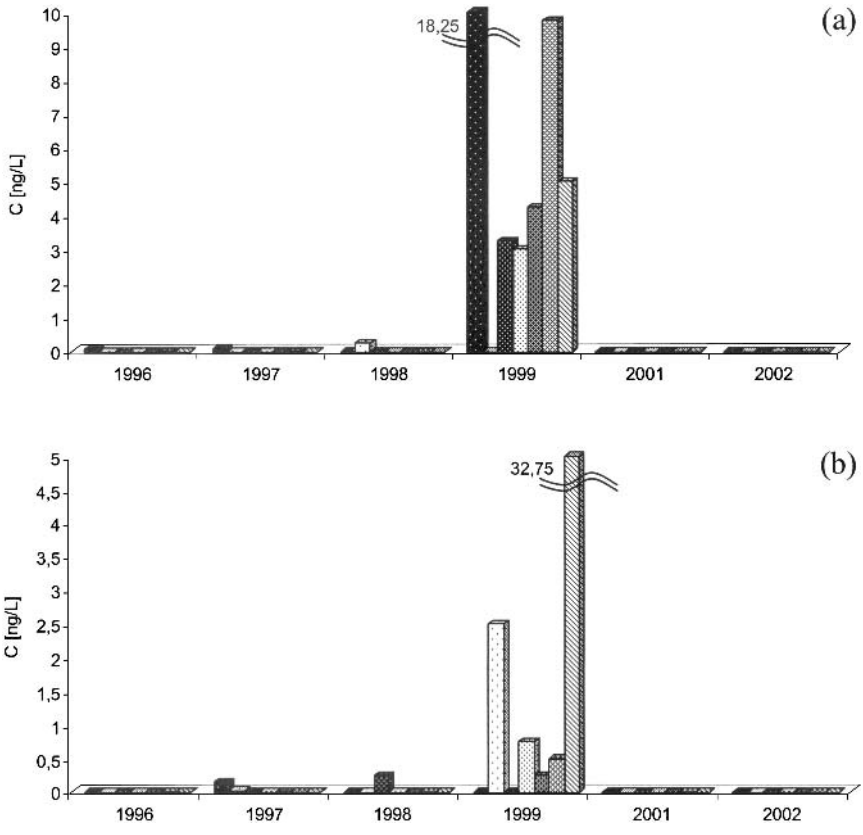

Fig. 11. Annual average concentrations of PCBs [ng/L] in the Gulf of Gdańsk and the Vistula River water in (a) Hel, (b) Władysławowo, (c) Orłowo, and (d) Kiezmark in 1996–2002.

DDT The use of DDT is banned in all countries around the Baltic, but this insecticide is still used to a great extent in tropical regions. It has earlier been demonstrated that the concentrations of DDT decreased rather rapidly after regulations and bans had been introduced in countries around the Baltic in the late 1970s. Eggshells of the guillemot have become thicker, but are still thinner than shells from guillemot eggs from preindustrial times. The effect of the DDT ban is clear with respect to the white-tailed sea eagle in the Baltic. After 25 years, the situation for the eagle has now returned to normal. Concentrations of DDT in fish (herring muscle tissue and cod liver) in the Baltic Proper have decreased substantially but are still higher than in fish from the Kattegat. There are also higher DDT concentrations in harbor seals in the Baltic Proper than in seals in the Kattegat area.

The residue of DDT and its metabolites (DDTs; *p*,*p*′-DDT, *o*,*p*′-DDT, *p*,*p*′-DDD, *o*,*p*′-DDD, *p*,*p*′-DDE, *o*,*p*′-DDE) has been determined in more than 10

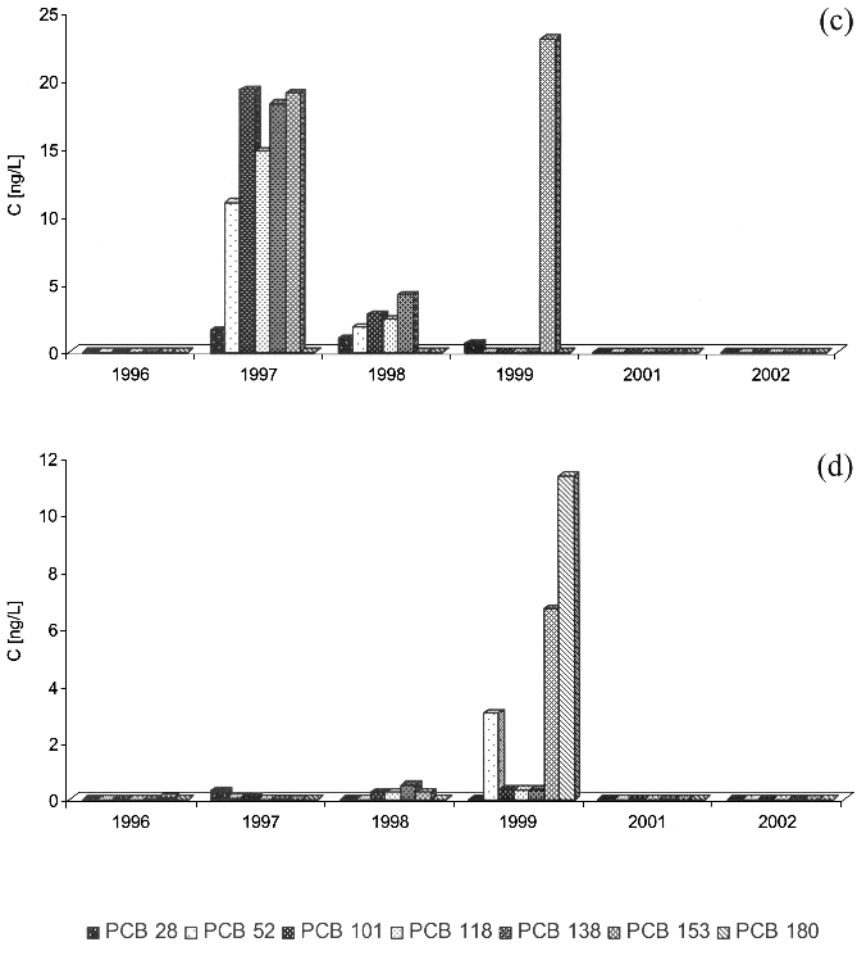

Fig. 11. *Continued.*

Table 7. Concentration of polychlorinated biphenyls (PCBs) in surface sediments.

Area	Sediment layer (cm)	ΣPCBs (ng/g dry wt)
Baltic Proper (12 sampling points)	—	Up to 11.0
Western (23) (22 sampling points)	0–3	<0.13–11.4
Gulf of Gdańsk (11 sampling points)	0–3	0.1–3.94 (particular PCBs)
Gulf of Gdańsk (13) (5 sampling points)	0–10 (each 1 cm)	1–9.5
Vistula Lagoon (6 sampling points)	—	0.1–0.99

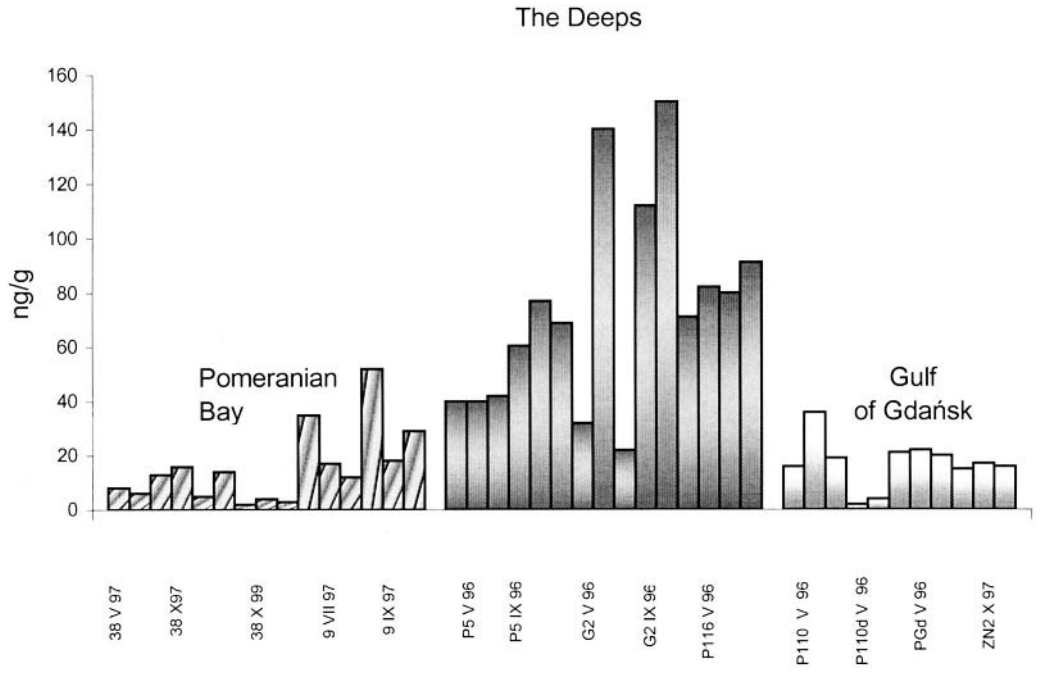

Fig. 12. Total content of PCBs in recent sediments of the Baltic Sea: 1996–1999, at each station in 0–1, 1–5, 5–10 cm layers in Odra Estuary; in 1997, in 0–2, 2–5, 5–10 cm layers. (From Renk 1993.)

species of edible fish caught in the Gulf of Gdańsk in 1992. Concentrations of specific DDTs in different fishes are shown in Table 9.

In 1996 the level of DDT in herring and in sprats was very high. In 1995 and 1997, lower levels were measured. The PCB content was similar to DDT (Falandysz et al. 1999). All fish examined (e.g., herring, cod, flounder, lamprey, perch, pike perch, round goby, eelpout) contained detectable residues of DDTs, and concentrations ranged from 28 to 310 ng/g wet weight. o,p′-DDT accounted for 0.4%–2.5% of DDT content. The concentration of DDTs in herring (110 ng/g wet weight and 1100 ng/g lipid weight) in 1992 was three times lower than in 1979–1983 and 14 fold lower than in 1969–1973 (Table 10).

E. PAHs

Changes in polycyclic aromatic hydrocarbon (PAH) concentration in water collected along the Polish coastal zones (measuring points are shown in Fig. 3) are illustrated in Fig. 14. PAHs occurred at the parts per thousand (ppt) level and the compounds most often detected were naphthalene, phenanthrene, benzo(*b*)-fluoranthene, and anthracene.

VI. Biological Conditions

Concentrations of biogenic compounds in the surface waters of the Basin of Gdańsk show extreme seasonal fluctuations; which is typical for the eutrophic environment. The maximum variations were noted in the inner part of the Gulf supplied with nutrients by the Vistula River and local sources. The large loads of nitrogen compounds result in rapid accumulation of nitrates in the coastal zone of the sea region and an increased ratio of assimilated nitrogen and phosphoros species by plants. Hence, the eutrophication and phytoplankton growth are accelerated. In consequence, the algae blooms cause recreation conditions to be unpleasant due to the offensive odor and taste. Some species present in the phytoplankton produce toxic compounds. In the shallow coastal sea areas, algae are damaging to other constituents of biocenoses by limiting their access to sunlight.

A. Pigment Composition

Numerous groups of compounds with diverse physical and chemical properties are included in pigments of phytoplankton organisms. Fundamentally, plant pigments are divided into three groups: chlorophylls (*a, b, c1, c2, c3*), carotenoids (carotenes and their oxygenated derivatives known as xanthophylls), and biliproteins (allophycocyanins, phycocyanins, hycoerythrins). All carotenoids are labile toward oxygen, light, heat, and acid. In the natural environment, the pigment composition may well vary with nutrient content and composition (Łotocka 1998; Stoń and Kosakowska 2000).

Concentrations of photosynthetic pigments in the sea are primarily dependent on the species composition and the photoadaptive state of the phytoplankton

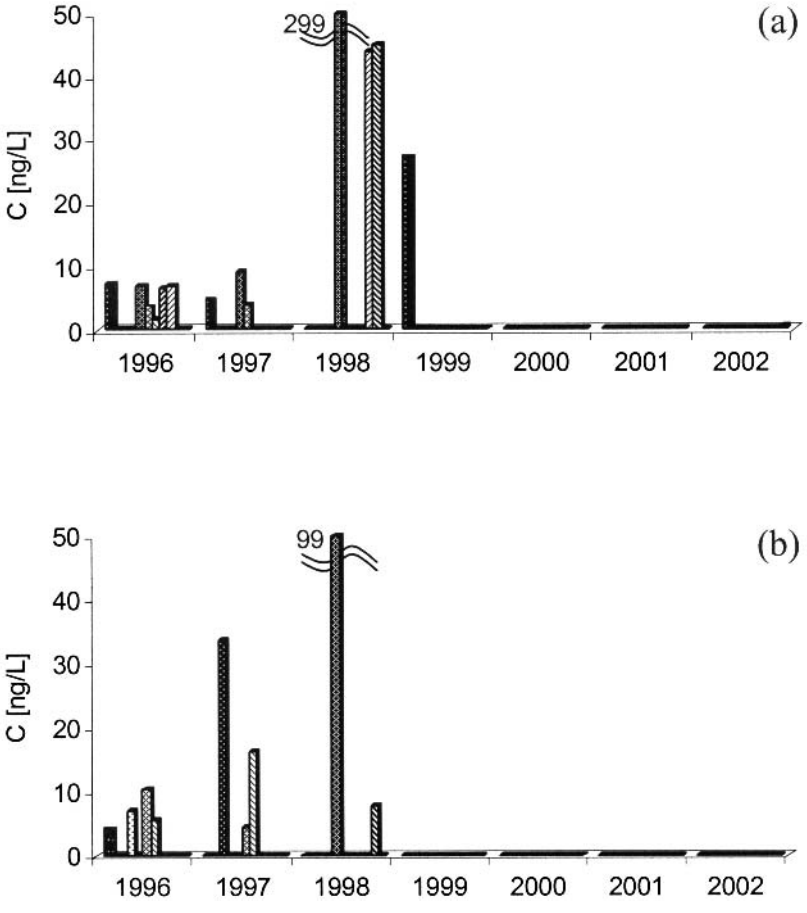

Fig. 13. Annual average concentrations of pesticides [ng/L] in the Gulf of Gdańsk and the Vistula River water in (a) Hel, (b) Władysławowo, (c) Orłowo, and (d) Kiezmark in 1996–2002.

present. Moreover, the pigment content of phytoplankton cells changes when the cells undergo photoacclimation or when they are exposed to stress conditions, such as limited access to light or iron.

The hydrological, environmental, and trophic conditions of the Gulf of Gdańsk are quite characteristic. From the geographic point of view it is part of the southern Baltic, a relatively shallow shelf sea with a limited exchange of water with the global ocean and a massive inflow of water from the Vistula River (Stoń and Kosakowska 2000).

The dominant groups of algae identified in the Gulf are dinoagellates, diatoms, chlorophytes, cyanobacteria, and other flagellates. The range of changes in concentration of pigments in natural samples of marine phytoplankton in

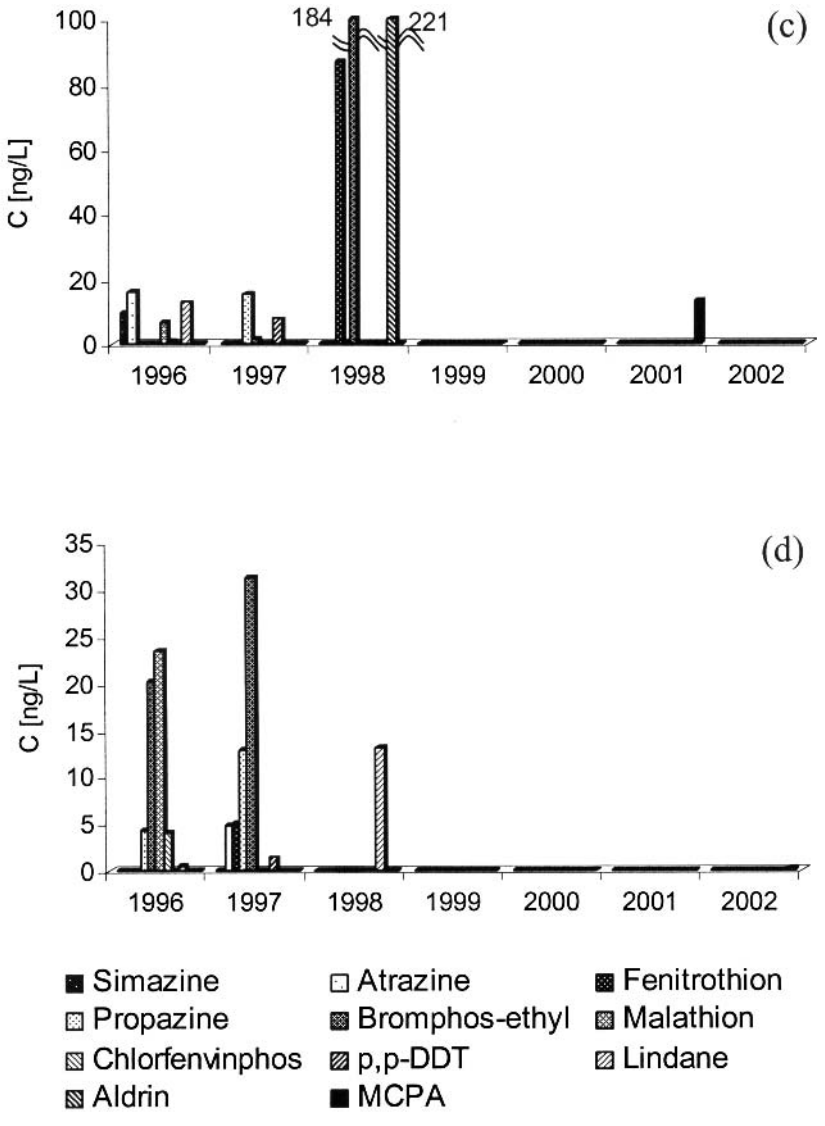

Fig. 13. *Continued*.

natural waters of the Gulf of Gdańsk (taken from sampling places from 18.50° N to 19.50° N and from 54.40° E to 54.80° E) is presented in Table 11.

Chlorophyll *a* has been observed in the Gulf in higher concentrations than the other pigments. A distribution of chlorophyll *a* in the upper surface layers of the Gulf of Gdańsk is shown in Fig. 15.

The photosynthetic carotenoids (peridinin, fucoxanthin, and α-carotene) are

Table 8. Annual loads of selected organochlorine compounds in the Baltic coastal zone (t/yr).

	1992	1993	1994	1995	1996	1997	1998
γ-HCH	0.3	0.4	0.3	0.5	0.5	—	—
DDE	0.2	0.2	0.1	0.1	0.1	—	—
DDD	0.1	0.1	0.03	0.03	0.01	—	—
DDT	0.3	0.2	0.1	0.1	0.2	—	—
DMDT	0.1	0.1	0.1	0.01	0.02	—	—

generally present in concentrations about one order of magnitude less than total chlorophylls. Peridinin and fucoxanthin, taxonomic biomarkers of dinoflagellates and diatoms, are identified in most of the samples (Stoń and Kosakowska 2000).

B. Phytoplankton

The first phytoplankton studies ever done in the Gulf of Gdańsk were undertaken just after World War II (Rumek 1948). However, these were concerned merely with species composition and seasonal changes. Later studies (Pliński et al. 1982, 1985) provided data on abundance and biomass of phytoplankton. Comparing the phytoplankton status of the 1970s and the 1980s to earlier periods (Rumek 1948), when data were restricted to species composition, its composition was found to vary from year to year. However, the domination of particular phytoplankton groups within a year was similar in the 1940s and the 1970s (Pliński et al. 1982, 1985). Seasonal occurrence of the phytoplankton blooms was influenced by a spring and autumn diatom bloom, the spring event being heavier and more differentiated than the autumn event, and a summer blue-green algae bloom. Up to the 1980s, the blue-green algae had been dominant in number. With regard to biomass, dinoflagellates were the most important, replacing the diatoms, which had dominated earlier.

Phytoplankton Species Composition In autumn 1994 the Gulf phytoplankton consisted of 47 taxa whereas in summer 1995 70 taxa were found, belonging to three phyla: Cyanophyta, Chlorophyta, and Chromophyta. During both seasons of investigation in the Gulf of Gdańsk coastal zone, phytoplankton species were noted that were classified as toxic or potentially toxic algae by Larsen and Moestrup (1989). Those species included the blue-green alga *Nodularia spumigena*, the dinoflagellate *Prorocentrum minimum*, and species of *Dinophysis* (*D. norvegica, D. acuminata, D. rotundata*).

Phytoplankton Abundance In 1994, phytoplankton quantity at the investigation station was within 2.2–8.8 billion units m^{-3}. A greater number was observed at the eastern end of the Gulf, with a broader range of values, between 2.67 and 24.37 billion units m^{-3}. The phytoplankton composition in autumn 1994 was

Table 9. Residue concentrations of DDTs, dieldrin, aldrin, endrin, and endosulfan 1 and 2 in fish from the Gulf of Gdańsk (ng/g wet wt).

Fish	Length (cm)	Lipids (%)	p,p′-DDT	o,p′-DDT	p,p′-DDD	o,p′-DDD	p,p′-DDE	o,p′-DDE	DDTs
Herring	16–21	9.7	11	0.6	43	2.0	53	nd	110
Cod	18–20	3.4	5.5	nd	22	0.22	35	0.06	70
Flounder	15–20	4.6	3.7	0.9	29	3.3	31	0.5	72
Lamprey	16–26	15.0	16	1.8	57	2.2	100	0.25	190
Perch	10–17	5.6	5.7	0.8	24	2.4	43	0.30	80
Pike perch	15–20	4.4	7.8	1.2	33	2.3	32	0.4	82
Round goby	16–26	4.8	1.2	0.3	11	1.2	27	0.2	40
Eelpout	23–27	3.0	5.5	0.5	19	1.2	110	0.7	140

Source: Falandysz et al. (1999).

Table 10. Concentrations of DDTs in herring in the Southern part of the Baltic Sea.

Year	ng/g (wet wt)	ng/g (lipid wt)
1980–1981	320 (150–560)	2500
1981	320 (280–370)	5900
1983	250 (59–1100)	3500
1985	380 (330–450)	6000
1986	200	5800
1987	190	4300
1992	100	1000

much poorer than in the summer of 1995, a characteristic feature of seasonal changes in phytoplankton. Comparing the Gulf of Gdańsk phytoplankton taxa numbers over many years, Pliński developed a hypothesis (Pliński et al. 1985) suggesting a diminishing abundance between 1946 and 1947. This hypothesis was based on the following: in the years 1946–1947, 260 taxa were found (Rumek 1948); in 1977–1978, 210 (Pliński et al. 1982); and in 1981, 213 taxa. It is worth mentioning that in 1994 the diatom number was several hundred times larger than the values determined in the 1970s (mean values increased from 0.6 million to 0.2 billion units m^{-3}), which also confirms the high eutrophication of the coastal zone of the Gulf of Gdańsk.

C. Biomass

Data from 1994 to 1998 collected for the southern Baltic Sea show that the mesozooplankton species composition did not change radically. From 1994 to 1998, populations of the halophilous copepod *Oithona similis* and *Pseudocalanus min. elongatus* were observed in the benthic waters of the Gdańsk Basin. The increase of biodiversity among Copepoda was related to the appearance of *Acartia tonsa* and *Limnocalanus grimaldii*, which occurred after a long-term absence. The greater species diversity of Copepoda corresponded with a simultaneous reduction of Cladocera species (Blachowiak-Samolyk and Opiola 2001).

Analyses of the average annual abundance of zooplankton in the past 5 yr indicate a strong relationship between Copepoda and Rotatoria abundance. This observation was especially evident in the Gdańsk Basin, where Copepoda dominated (in 1994, 1996, and 1998) alternately with Rotatoria (1995 and 1997). This phenomenon was also observed in the Gotland Basin, where the most spectacular example occurred in 1995, when Rotatoria constituted almost 70% of the zooplankton abundance. This event was due to the unusually long occurrence of *Synchaeta* sp., a thermophilous species, which probably resulted from the higher water temperature. Large variations in the annual abundance from 1979 to 1998 allowed the cycles of changes in mesozooplankton abundance to be seen (Blachowiak-Samolyk and Opiola 2001).

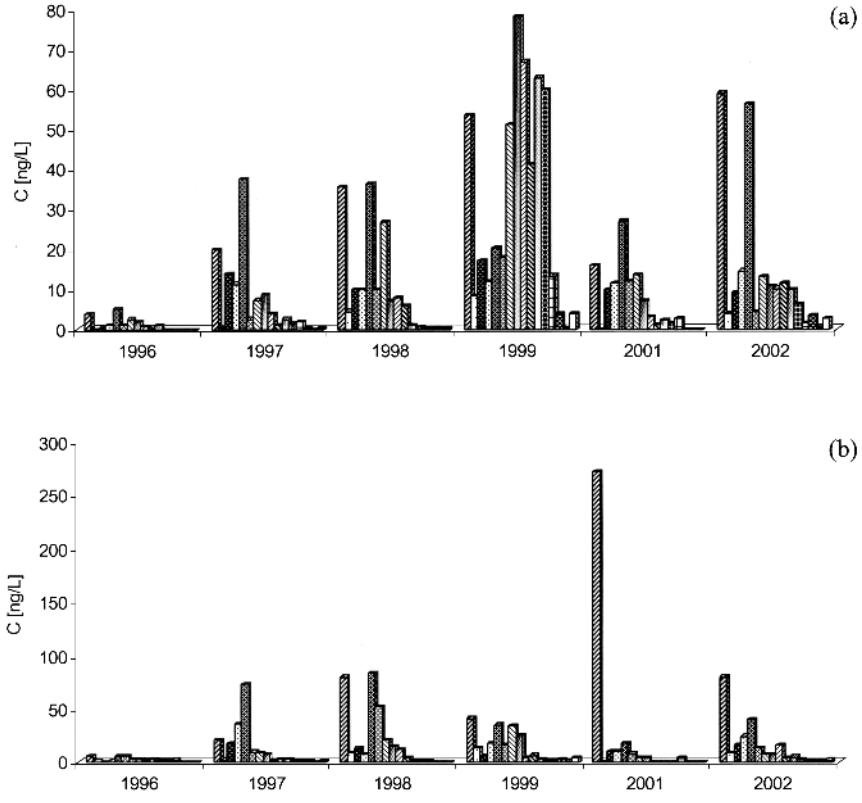

Fig. 14. Annual average concentrations of PAHs [ng/L] in the Gulf of Gdańsk and the Vistula River water in (a) Hel, (b) Władysławowo, (c) Orłowo, and (d) Kiezmark in 1996–2002.

Analyses of zooplankton biomass reveal that there are certain rules that repeat in annual and long-term cycles. Seasonal changes in zooplankton biomass depend mainly on the seawater temperature. The increase of biomass after winter occurs in April and reaches its maximum value in July–August. After September, the zooplankton biomass decreases to minimum values in winter.

A significant decrease in zooplankton biomass was observed over the 5-yr period 1994–1998. In 1998, the biomass was three times lower than in 1994, which was directly related to a significant decrease in phytoplankton abundance over the 1995–1998 period (Blachowiak-Samolyk and Opiola 2001).

VII. DPSIR Proposal for the VisCat Case

The pace and extent of changes that occur in the catchment-coast system are controlled by increasingly complex relationships between biogeochemical and socioeconomic parameters. To overcome the complexity of the analysis, within

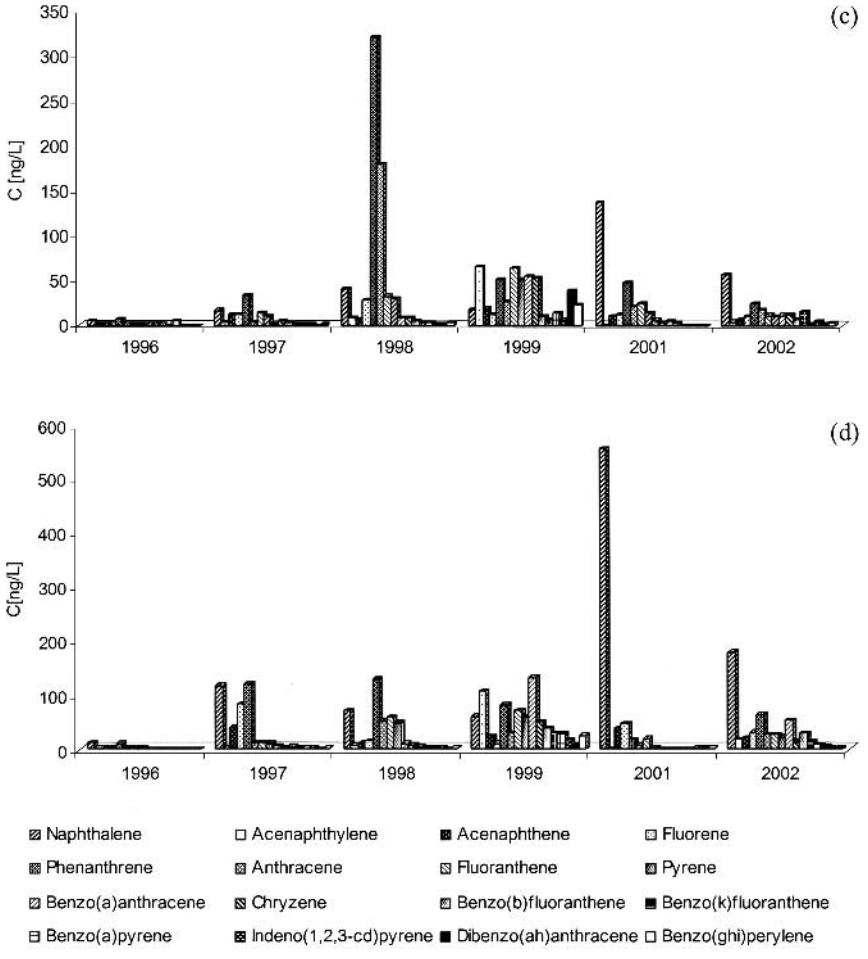

Fig. 14. *Continued.*

the EUROCAT project (EVK1-CT-2000-00044), a simplified organizational and auditing framework, the Driver–Pressure–State–Impact–Response (DPSIR), has been adopted to underpin the regional studies and allow integration at the European level.

DPSIR is a multiple feedback framework based on the assumption that the continuum catchment-coast acts as a dynamic system whose changes are strictly connected to the socioeconomic issues affecting the catchment area. Environmental pressures originated by socioeconomic forces (drivers), demographic, economic, institutional, and technological, cause significant environmental state changes in the catchment-coast system. These changes include increased nutrient, sediment, and pollutant loads across the drainage systems and in the marine

Table 11. The range of changes in concentration of pigments (μg dm^{-3}) in natural samples of marine phytoplankton.

	April 1999		September 1999	
Pigment	Minimum	Maximum	Minimum	Maximum
Chlorophyll *a*	0.43 (30 m)	91.79 (0 m)	0.05 (20 m)	11.59 (0 m)
Chlorophyll $c_1 + c_2$	0.04 (30 m)	11.79 (0 m)	0.02 (20 m)	0.88 (0 m)
Chlorophyll *b*	0.06 (20 m)	1.68 (3 m)	0.16 (0 m)	1.18 (0 m)
Fucoxanthin	0.015 (30 m)	6.31 (3 m)	0.02 (20 m)	0.43 (0 m)
Peridinin	0.05 (30 m)	19.31 (0 m)	0.11 (7 m)	1.13 (0 m)
α-Carotene	0.06 (10 m)	0.58 (0 m)		
Diadinoxanthin	0.022 (30 m)	9.166 (0 m)	0.026 (20 m)	0.71 (0 m)
Alloxanthin	0.034 (40 m)	3.41 (0 m)	0.018 (20 m)	0.198 (0 m)
Zeaxanthin	0.02 (40 m)	0.24 (0 m)	0.04 (7 m)	1.23 (0 m)
β-Carotene	0.099 (10 m)	1.27 (0 m)	0.18 (5 m)	0.49 (0 m)
Lutein	0.07 (0 m)	0.93 (15 m)		
Neoxanthin	0.02 (0 m)	0.21 (3 m)	0.038 (5 m)	0.128 (0 m)
Violaxanthin	0.09 (0 m)	0.51 (3 m)	0.007 (5 m)	0.28 (0 m)

Source: Stoń and Kosakowska (2000).

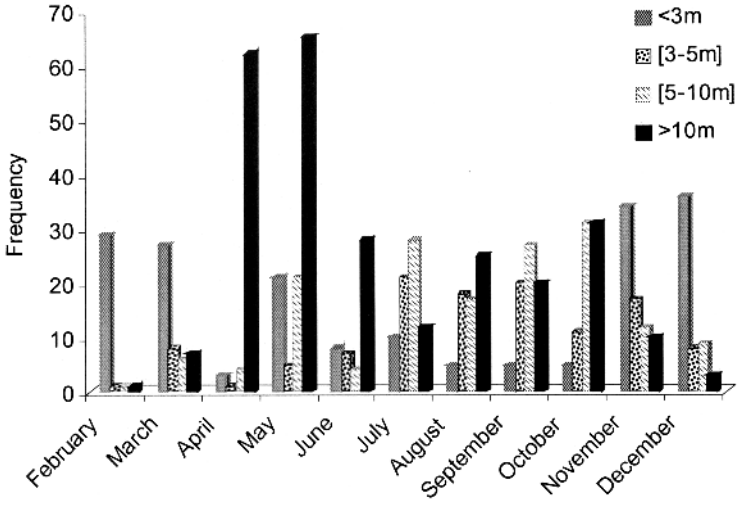

Fig. 15. Chlorophyll *a* content [mg m^{-3}] distribution in the surface layer of the Gulf of Gdańsk [based on the measurements of Latala (1993, 1996) in 1986, 1987, 1992–1994]. (From Krezel and Latala 1999.)

environment; alteration of river flow regimens; habitat fragmentation and degradation; loss of biodiversity; soil, water, and atmospheric pollution; and eventually climate changes. The severity of some of the resulting damages is worsened because of the variability (nature and human-induced) of coastal processes. When the processing and functioning capabilities of ecosystems are affected, welfare losses occur as a result of changes in productivity, health, amenity, and other values (impacts). Policy response mechanisms should then be triggered to overcome the observed impacts, in a continuous and adaptive feedback process aimed to reestablish a balance between human activities and natural resources.

The DPSIR proposal for the VisCat case taking Vistula River and Gulf of Gdańsk as a catchment/coastal area is graphically presented in Fig. 16.

VIII. Conclusions

The present pollution of the Gulf of Gdańsk is mainly the effect of the past, when enormous amounts of pollutants were discharged via either rivers or atmosphere. Because degradation processes of the environment in the area of the Gulf of Gdańsk (Baltic Sea) are continuous and long term, development there is a necessity of permanent control and improvement of the conditions in the Gulf. This control is of vital importance for the future survival of the Baltic.

Summary

This review presents the present state of knowledge of the physical, chemical, and biological changes in waters of the Gulf of Gdańsk (Baltic Sea). The general characteristics of the Baltic Sea and the Gulf of Gdańsk with brief description of changes in the ecosystem are included. Among meteorological parameters describing climate in the coastal zone, water temperature changes together with considerations of precipitation and sea level are presented. It has been confirmed that the sea level rise over the past 40 yr was very distinct. Throughout the past 30 yr an evident increase in eutrophication has been observed. Therefore, changes in salinity, oxygen content, and nutrient fluctuations with special attention paid to variability in silicate, nitrate, and phosphate concentrations in the water layer are presented. Also, discussion on the presence of toxic pollutants, such as heavy metals, PCBs, PAHs, and some pesticides, in the water body of the Gulf of Gdańsk has been included. Because of their ability to bioaccumulate and biomagnify in living organisms, these substances are of crucial importance for future marine life in the Gulf of Gdańsk. Finally, biological conditions of the coastal waters of the Gulf of Gdańsk were discussed. Data show that the ecosystem structure of the Gulf of Gdańsk has undergone tremendous changes. The species pattern has changed significantly—many species have disappeared and others have become dominant.

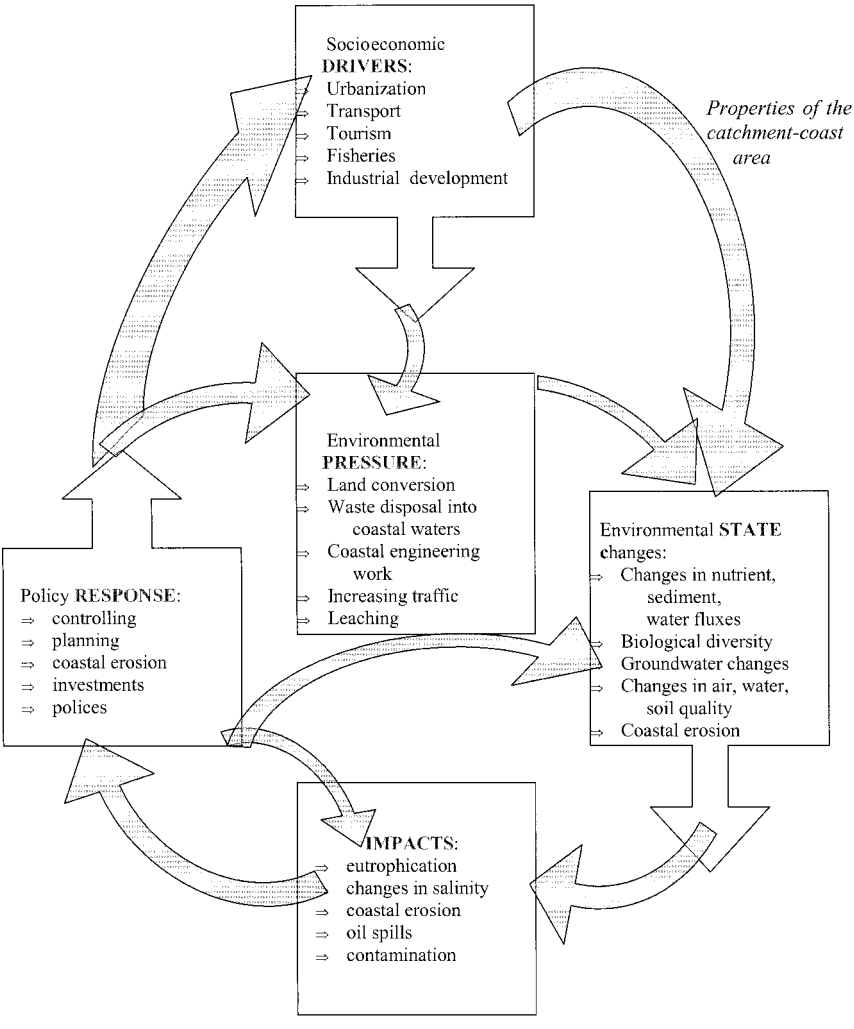

Fig. 16. DPSIR proposal for the VisCat case taking Vistula River and Gulf of Gdańsk as a catchment/coastal area.

Acknowledgments

This article was financially supported by EuroCat project EVK1-CT-2000-00044 (European Community) and a grant 155/E-359/SPUB-M/5PRUE/Dz 164/2004 2001-2002 and 8T07G 00621 (Polish Committee of Scientific Research). We thank B. Zygmunt, M. Biziuk, A. Wasik, and R. Kartanowicz for their contribution in the preparation of this manuscript.

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Manuscript received November 18; accepted November 26, 2002

Rev Environ Contam Toxicol 179:37–71

Spinosad Toxicity to Pollinators and Associated Risk

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and Mark M. Miles

Contents

I. Introduction

Spinosad is a natural insecticide derived from a species of actinomycete bacterium, *Saccharopolyspora spinosa* (Mertz and Yao 1990), that displays the efficacy of a synthetic insecticide. Fermentation of *S. spinosa* produces several metabolites that have been designated spinosyns. Spinosad consists of the two most biologically active spinosyns, A and D. The first approved use of spinosad

Communicated by George W. Ware.

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in the United States was in 1997 as a reduced-risk insecticide on cotton. Spinosad is sold in more than 50 countries under the tradenames of Tracer[1], Success[1], SpinTor[1], Spinoace[1], Conserve[1], GF-120 Naturalyte Fruit Fly Bait, and others. Typical application rates range from 25 to 150 g a.i./ha with 75 g a.i./ha being an average for most uses. The majority of the product is sold as a suspension concentrate (SC). Spinosad SCs contain spinosad milled to 5–10 μm in an aqueous suspension that lacks organic solvents. Depending on customer and crop needs, spinosad SCs are provided in concentrations ranging from 120 to 480 g/L. Spinosad is also marketed as water-dispersible granule (WDG) formulations. These dry WDG formulations must be mixed with water and applied similarly to the SC formulations. Once diluted and mixed in water, they form suspensions that are identical to the SCs in particle size and residues on plant surfaces. The GF-120 Naturalyte[1] Fruit Fly Bait formulation is unique. The bait contains sugar-based feeding stimulants and produces amino compounds that are attractive to fruit flies but not honeybees. It is diluted in water to contain 20–80 ppm of spinosad and, because it attracts the fruit flies to the bait, it is applied at a very low rate, <0.5 g a.i./ha.

Spinosad's rapid acceptance as an alternative to older insecticides can be attributed to its low use rate, efficacy, and broad spectrum of activity on key pests, novel mode of action, favorable environmental and toxicological profile, and its suitability for integrated pest management strategies. Existing literature addresses these attributes and includes the discovery, physical and biological properties of spinosad (Mertz and Yao 1990; Kirst et al. 1992; DeAmicis et al. 1997; Sparks et al. 1998; Thompson and Hutchins 1999), mode of action and structure activity relationships (Crouse and Sparks 1998; Salgado 1998; Salgado et al. 1997, 1998; Watson 2001), basic chemistry of spinosyns (Kirst et al. 1992), efficacy (Peterson et al 1997), environmental fate (Thompson and Hutchins 1999; Reeves et al. 1996; Tomkins et al. 1999; Cleveland et al. 2002; Saunders and Bret 1997), and mammalian and ecotoxicology (Breslin et al. 2000; Cleveland et al. 2001; Stebbins et al. 2002; Yano et al. 2002).

II. Physiochemical Properties

Spinosad is a large, complex molecule built around a central (aglycone) ring system. (Fig. 1). Spinosad is a mixture of spinosyn A [2-((6-deoxy-2,3,4-tri-*O*-methyl-alpha-L-mannopyranosyl)oxy)-13-((5-dimethylamino)tetrahydro-6-methyl-2*H*-pyran-2-yl)oxy)-9-ethyl-2,3,3a,5a,5b,6,9,10,11,12,13,14,16a,16b-tetradecahydro-14-methyl-1*H*-as-indaceno(3,2-d)oxyacyclododecin-7,15-dione], and spinosad D [2-((6-deoxy-2,3,4-tri-*O*-methyl-alpha-L-mannopyranosyl)oxy)-13-((5-(dimethylamino)tetrahydro-6-methyl-2*H*-pyran-2-yl)oxy)-9-ethyl-2,3,3a,5a,5b,6,9,10,11,12,13,14,16a,16b-tetradecahydro-4,14-dimethyl-1*H*-as-indaceno(3,2-d)oxyacyclododecin-7,15-dione], and behaves as a hybrid of the two factors. Although there are some differences in the physicochemical properties between

[1]Trademark of Dow AgroSciences LLC.

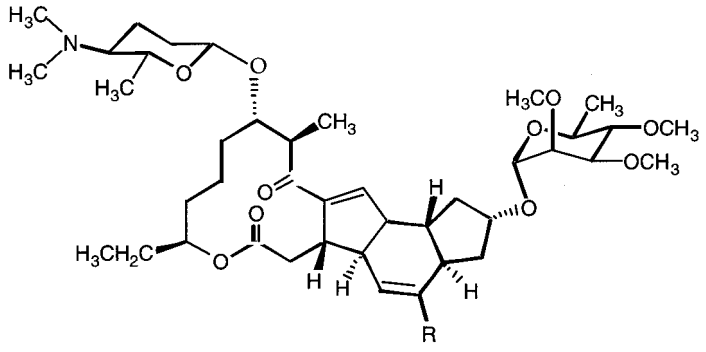

Fig. 1. Structure of spinosad: spinosyn A (R = H) and spinosyn D (R = CH_3).

individual factors, the two factors are more similar than distinct (Saunders and Bret 1997; Thompson and Hutchins 1999). Spinosad in nonvolatile (vapor pressures range from 2.1 to 3.2 E^{-8} Pa), is positively charged at environmental pH, and is nonpolar (log *P* values range from 2.8 to 5.2). The solubility of the respective spinosyns in water differs. At pH 7 and 20 °C, water solubility is 235 mg/L for spinosyn A and 0.332 mg/L for spinosyn D. For the more soluble spinosyn A, K_{ads} (adsorption coefficient) and K_{des} (desorption coefficient) values are calculated to be 8.3–323 mL/g. These coefficients suggest that spinosad will partition to and be strongly sorbed to organic matter.

III. Environmental Fate

Both spinosyn A and spinosyn D are readily degraded by soil microbial action (Saunders and Bret 1997; Thompson and Hutchins 1999). Studies under controlled laboratory conditions indicate a biphasic degradation pattern with a half-life of the order of 2–3 wk. Spinosyn A incubated aerobically in moist soil (75% 1/3 bar), held in the dark at 25°C, exhibited half-lives of 9.4–17.3 d. The half-life of spinosyn D under identical incubation conditions was 14.5 d. However, field dissipation studies indicate much shorter half-lives of 0.3 and 0.5 d for spinosyn A and D, respectively. The rapid dissipation of spinosad in soil and plant foliage following field application can be attributed to photolysis or a combination of metabolism and photolysis (Saunders and Bret 1997). The low vapor pressure of spinosyns A and D precludes volatilization from plant surfaces or soil, and thus is not a route of dissipation in the environment. Principal transformation products of spinosyns A and D are N-demethylation products designated as spinosyn B and N-demethylated spinosyn D. Neither is considered to be of toxicological concern.

IV. Mode of Action in Insects

Extensive studies on the insecticidal mode of action of spinosad have been conducted using a variety of physiological and biochemical approaches (Salgado 1998; Watson 2001; N. Orr 2002, Dow AgroSciences, personal communica-

tion). These studies suggest that spinosad exerts its insecticidal actions via a novel mode of action (i.e., not previously reported for other insecticides). Exposure of insects to spinosad results in events characterized by initial involuntary muscle contractions and tremors, followed by paralysis and death (Salgado et al. 1997; Salgado 1998). All these effects are consistent with hyperexcitation of the insect nervous system. Ganglia from insects exposed to spinosad exhibit intense neuronal firing, followed by inhibition of firing (Salgado et al. 1997; Salgado 1998). Further characterization of these effects has led to a hypothesis of effects on novel nicotinic receptors or currents, as well as some effects on GABA-gated chloride currents (Watson 2001). Interestingly, there does not appear to be direct receptor interaction with any of the known, insecticidally relevant receptor sites (e.g., neonicotinoids and avermectins) (N. Orr, 2002, Dow AgroSciences, personal communication). These data suggest that spinosad interacts via some novel mechanism that alters or activates both nicotinic and GABAergic currents in an insect-selective manner.

V. Nontarget Toxicity

Spinosad has a high level of efficacy for lepidopteran larvae, as well as some Diptera, Coleoptera, Thysanoptera, and Hymenoptera, but has limited or no activity against other insects (Thompson and Hutchins 1999), and exhibits low toxicity to mammals and other wildlife (Cleveland et al. 2001). Although spinosad has low toxicity to most beneficial insects, initial acute laboratory tests indicated that spinosad is intrinsically toxic to pollinators. Based on initial Tier 1 tests, the U.S. Environmental Protection Agency initially required a label warning regarding spinosad toxicity to bees. Higher-tier tests expand on the initial laboratory bioassays by assessing effects under more natural conditions. In recognition of the significant economic and social importance of pollinators, a large number of higher-tier studies have been conducted to assess the hazard of spinosad to pollinators. This review examines recent Tier 1 and higher-tier studies conducted to assess the hazard of spinosad to bees and provides an assessment of the risk of spinosad to pollinators under actual field conditions.

VI. Hazard Evaluation

A number of laboratory tests and semifield and field trials were conducted in the United States, Europe, and the Pacific to assess the hazard of spinosad to bees. The protocols and experimental designs for these investigations differed, but the objective of each was to assess the potential effects of spinosad on pollinators. These investigations are reviewed here in an iterative manner with early-tier laboratory studies presented first, followed by higher-tier studies including greenhouse, semifield, and full field evaluations.

A. Acute Laboratory Tests

Acute laboratory tests were conducted with pollinators to assess the intrinsic toxicity of technical spinosad (88% active ingredient, a.i.), a 480 g a.i./L SC, and a 0.02% a.i. fruit-fly bait. Tests were conducted following either FIFRA Guideline Series 141-1 (U.S. EPA 1982) or EPPO Guideline No. 170 (European Plant Protection Organization 1992). Bees were exposed orally (in sugar water diet) or topically. Mortality and sublethal effects were recorded at 24 or 48 hr after treatment (Table 1).

Acute Contact Toxicity Studies were conducted to assess the acute contact toxicity of technical spinosad to the honeybee (*Apis mellifera* L.). Hoxter et al. (1992) reported the 48-hr LD_{50} to be 0.0025 µg/bee with a no observed effect concentration (NOEC) of 0.0016 µg/bee. A more recent study (Halsall and Grey 1998a) reported the 48-hr LD_{50} to be 0.04 µg a.i./bee and reported mortality of 3.3% at 0.025 µg/bee. For toxicity of technical spinosad to the honeybee, alfalfa leafcutter bee (*Megachile rotundata* (F.), and alkali bee (*Nomia melanderi* Cockerell), the LD_{50} values were reported to be 0.078, 0.058, and 0.065 µg/bee, respectively (Mayer et al. 2001). These data are in agreement with those of Halsall and Gray (1998a), who reported a 24-hr LD_{50} for the honeybee of 0.057 µg/bee.

The acute contact toxicity of the 480 g a.i./L SC to honeybees was also evaluated (Halsall and Grey 1998b; Perina 1996). Halsall and Grey (1998b) reported the 24- and 48-hr LD_{50} values as 0.16 and 0.12 µg product/bee, respectively. Perina (1996) reported the 24-hr LD_{50} as 1.84 µg product/bee. The contact toxicity of a fruit-fly bait, 0.2 g a.i./L, was determined with the honeybee (Hahne 2000). The 24- and 48-hr LD_{50} values were reported as >100 µg product/bee. There was 17% mortality at the 100 µg/bee test level; however, mortality at 50 µg formulation/bee and lower was similar to that of the controls.

Contact toxicity of 240 g a.i./L SC to the honeybee, alfalfa leafcutter bee, and alkali bee was also assessed using a less conventional procedure (Mayer et al. 2001). Aqueous preparations were sprayed on bees using a CO_2 pressurized microsprayer. Groups of 30 bees were sprayed with 0.45 mL of five different concentrations; the control was treated with 0.45 mL of water. The 24-hr LD_{50} was reported as 311, 251, and 510 mg/L (equivalent to 1.1, 0.9, and 1.8 µg/bee, respectively) for the honeybee, alfalfa leafcutter bee, and alkali bee, respectively. Aldershof (1999a) investigated the acute contact of the 480 g/L SC to the bumblebee (*Bombus terrestris* L.) and reported the 48- and 72-hr LD_{50} to be 19.4 and 15.5 µg a.i./bee, respectively.

Acute Oral Toxicity The oral toxicity of spinosad to bees has also been investigated. The 48-hr oral LD_{50} for honeybees exposed to the technical material was determined to be 0.053 µg a.i./bee, and the 48-hr oral LD_{50} of 480 g a.i./L SC was determined to be 0.11 µg product/bee (Halsall and Grey 1998a,b). A similar study using 480 g a.i./L SC with the bumblebee found the 48- and 72-hr LD_{50} to be 0.39 and 0.34 µg a.i./bee, respectively (Aldershof 1999b). These data

Table 1. Acute contact and oral tests with bees.

Test type	Test material	Method	Test results	Toxicity classification	Reference
Honeybee, acute contact	Technical (88% a.i.)	Direct application to bee	48 hr LD_{50}, 0.0025 µg a.i./bee	Highly toxic	Hoxter et al. 1992
Honeybee, acute contact	Technical (88% a.i.)	Direct application to bee	48 hr LD_{50}, 0.04 µg a.i./bee	Highly toxic	Halsall and Grey 1998a
Honeybee, acute contact	Technical (88% a.i.)	Direct application to bee	24 hr LD_{50}, 0.078 µg a.i./bee	Highly toxic	Mayer et al. 2001
Alfalfa leafcutter bee, acute contact	Technical (88% a.i.)	Direct application to bee	24 hr LD_{50}, 0.058 µg a.i./bee	Highly toxic	Mayer et al. 2001
Alkali bee, acute contact	Technical (88% a.i.)	Direct application to bee	24 hr LD_{50}, 0.065 µg a.i./bee	Highly toxic	Mayer et al. 2001
Honeybee, acute contact	480 g a.i./L SC	Direct application to bee	48 hr LD_{50}, 0.12 µg formulation/bee	Highly toxic	Halsall and Grey 1998b
Honeybee acute contact	480 g a.i./L SC	Direct application to bee	24 hr LD_{50}, 1.843 µg formulation/bee	Moderately toxic	Perina 1996
Honeybee acute contact	0.2 g a.i./L Fruit Fly Bait	Direct application to bee	48 hr LD_{50}, >100 µg formulation/bee	Nontoxic	Hahne 2000
Honeybee, acute contact	240 g a.i./L SC	Microsprayer application	24 hr LD_{50}, 311 µg a.i./mL (1.1 µg a.i./bee)	Highly toxic	Mayer et al. 2001

Table 1. (*Continued*).

Test type	Test material	Method	Test results	Toxicity classification	Reference
Alfalfa leafcutter bee, acute contact	240 g a.i./L SC	Microsprayer application	24 hr LD_{50}, 251 µg a.i./mL (0.9 µg a.i./bee)	Highly toxic	Mayer et al. 2001
Alkali bee, acute contact	240 g a.i./L SC	Microsprayer application	24 hr LD_{50}, 510 µg a.i./mL (1.8 µg a.i./bee)	Highly toxic	Mayer et al. 2001
Bumblebee, acute contact	480 g a.i./L SC	Direct application to bee	48 hr LD_{50}, 19.4 µg a.i./bee	Moderately toxic	Aldershof 1999a
Honeybee, acute oral	240 g a.i./L SC	Oral presentation in sucrose	24 hr LC_{50}, 0.063 µg a.i./bee	Highly toxic	Mayer et al. 2001
Honeybee, acute oral	Technical (88% a.i.)	Oral presentation in sucrose	48 hr LC_{50}, 0.053 µg a.i./bee	Highly toxic	Halsall and Grey 1998a
Honeybee, acute oral	480 g a.i./L SC	Oral presentation in sucrose	48 hr LC_{50}, 0.11 µg formulation/bee	Highly toxic	Halsall and Grey 1998b
Bumblebee, acute oral	480 g a.i./L SC	Oral presentation in sucrose	48 hr LC_{50}, 0.385 µg formulation/bee	Highly toxic	Aldershof 1999b

SC: suspension concentrate.

demonstrate that technical spinosad in highly toxic to bees when applied directly to bees or when provided in the diet. Lesser toxicity is observed with dilute SC formulations and the highly dilute fruit fly formulation.

B. Studies to Evaluate the Toxicity of Dislodgeable Residues

These studies were designed to address a more realistic exposure scenario by evaluating the toxicity of spinosad either by dermal contact to, or ingestion of, dried residues on plant foliage, rather than direct application. The experimental design represents a highly conservative scenario because the bees are confined to the treated substrate. A summary of these data is provided in Table 2.

Alfalfa The toxicity of spinosad residues on alfalfa to the honeybee was evaluated in two studies. Applications of 240 g a.i./L SC were made to alfalfa plots at a rate of 43 g a.i./ha in an application volume of 935 L/ha (Palmer and Krueger 1997) or 177 g a.i./ha in 48 L/ha (Kranzfelder 1999). The application volumes represent those used for trees and vines and for a vegetable, respectively. Replicate alfalfa plots were sprayed at 3, 8, or 24 hr before harvest. Following harvest, alfalfa foliage from each treatment or control was chopped and placed into replicate test chambers. Worker honeybees were introduced into each test chamber and maintained under controlled conditions for 24 hr. Bees were observed for mortality and signs of toxicity. The application of spinosad at 43 or 177 g a.i./ha to alfalfa in a volume of either 935 or 48 L/ha and allowed to weather for 3, 8, or 24 hr did not result in significant bee toxicity when compared to the controls (Table 3). These data demonstrate the low potential for honeybee mortality following 3 hr weathering of residues and that the hazard of dried spinosad residues to bees is not dependent on application volume.

The toxicity of spinosad residues to the honeybee, alfalfa leafcutter bee, and alkali bee was evaluated by Mayer et al. (2001). Three formulations [10 g/kg WP (wettable powder), 800 g/kg WDG, and 240 g/L SC] were applied to small plots of alfalfa at 50, 100, and 200 g a.i./ha using a pressurized sprayer. Samples of alfalfa with field-weathered residues were collected 2 and 8 hr after treatment and placed in cages with bees. Mortality was assessed after 24 hr of exposure. Irrespective of treatment, the 2-hr and 8-hr residues did not affect the honeybee. There was a notable effect on both the alfalfa leafcutter bee and the alkali bee, with the effect generally reduced in groups exposed to residues weathered for 8 hr. The alkali bee was marginally more tolerant than the alfalfa leafcutter bee (Table 4).

Kiwifruit The toxicity of 120 g a.i./L SC applied at 72 or 144 g a.i./ha to honeybees exposed to sprayed staminate flowers of kiwifruit (*Actindia chinensis* Planch) was evaluated by Goodwin and Haine (1998) (see Table 2). Tau-fluvalinate[2] was used as a nontoxic reference and applied at 72 g a.i./ha. All treatments

[2]The chemical name and CAS registration numbers for other insecticides mentioned in the text appear in the Appendix.

Table 2. Laboratory residue studies on foraging bees.

Test type	Test material	Method/crop	Application rate	Effects description	Reference
Toxicity of residues to the honeybee	240 g a.i./L SC	Laboratory exposure to plot-treated alfalfa	42 g a.i./935 L/ha	Nontoxic following 3 hr weathering	Palmer and Krueger 1997
Toxicity of residues to the honeybee	240 g a.i./L SC	Laboratory exposure to plot-treated alfalfa	177 g a.i./48 L/ha	Nontoxic following 3 hr weathering	Kransfelder 1999
Toxicity of residues to the honeybee	120 g a.i./L SC	Laboratory exposure to treated kiwifruit flowers	72 or 144 g a.i./ 1500 L/ha	Dried residues nontoxic	Goodwin and Haine 1998

Table 3. Mortality of honeybees exposed to weathered spinosad residues.

Weathering interval on alfalfa	24-hr mortality, dead/exposed (% mortality)	
	42 g a.i./ha: volume, 935 L/ha	177 g a.i./ha: volume, 48 L/ha
Control, 3 hr	5/450 (1)	6/150 (4)
Spinosad		
3 hr	28/450 (6)	5/150 (3)
8 hr	23/450 (5)	0/150 (0)
24 hr	11/450 (2)	2/150 (1)

were applied in a volume of approximately 1500 L/ha. Open staminate flowers were picked and placed on a tray and subjected to one of the described treatments. After drying, flowers were placed on the floor of cages (10 replicates per treatment), followed by 20 adult honeybees. The number of dead bees was assessed after 1, 16, 24, 40, 48, 63, 73, 89, and 100 hr. Following exposure to treated kiwifruit flowers, there was no significant difference ($P < 0.05$) in bee mortality between any of the treatments and the control except at one time point. During the 16-hr assessment, the tau-fluvalinate treatment and the control group had significantly higher mortality than the spinosad (144 g a.i./ha) treatment. Mortality data are presented in Fig. 2. These data further demonstrate that spinosad residues are not toxic to honeybees provided that the residues are allowed to dry before the bees forage.

Table 4. Mortality (%) of bees following exposure to weathered spinosad residues on alfalfa.

Treatment	Application rate, g a.i./ha	Honeybee		Alfalfa leafcutter bee		Alkali bee	
		2 hr	8 hr	2 hr	8 hr	2 hr	8 hr
10 g/kg WP	50	4	0	10	13	9	7
10 g/kg WP	100	0	3	12	5	10	2
10 g/kg WP	200	0	0	36	26	25	21
800 g/kg WDG	50	0	0	18	5	8	8
800 g/kg WDG	100	0	0	10	12	14	7
800 g/kg WDG	200	2	0	38	29	28	17
240 g/L SC	50	0	0	12	8	7	6
240 g/L SC	100	3	0	12	12	16	3
240 g/L SC	200	0	0	31	24	29	7

WP: wettable powder; WDG: water-dispersible granule; SC: suspension concentrate.

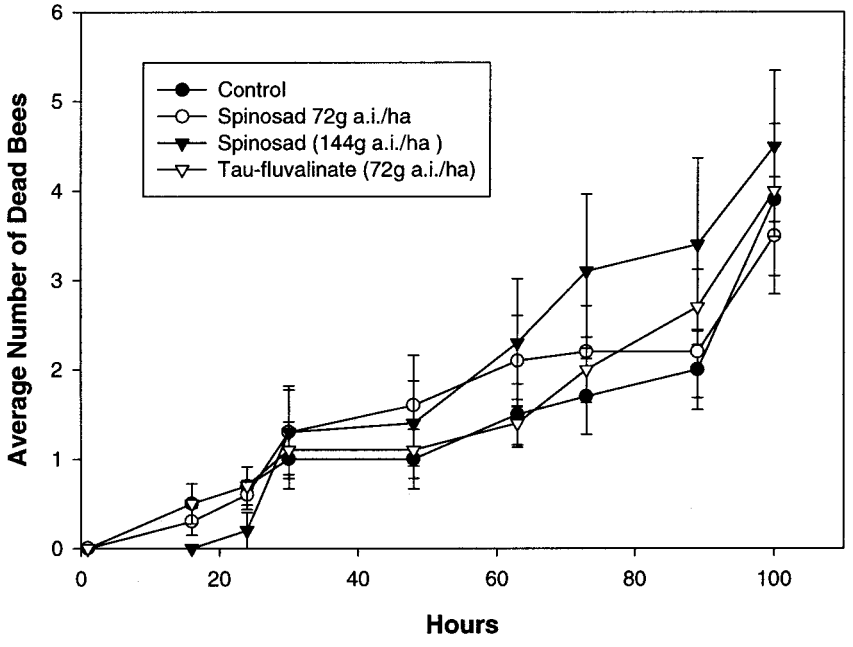

Fig. 2. Mortality of bees exposed *in vitro* to kiwifruit flowers treated with spinosad and tau-fluvalinate.

C. Greenhouse Studies

These studies were designed to evaluate the effect of spinosad on pollinators under typical greenhouse conditions. Two studies were conducted to assess the effect of spinosad on bumblebees following application of spinosad (SC and WDG formulation) to tomatoes, and one study was conducted with honeybees following a WDG formulation application to strawberries (Table 5.)

Tomatoes Spinosad (480 g a.i./L SC) was applied to tomatoes to assess the effects of foliar application of spinosad on foraging bumble bees (Aldershof 2000). Mortality, foraging activity, and brood development were compared to an imidacloprid treatment and an untreated control. Methods followed the recommendations of Barrett et al. (1994). Spinosad was applied in an application volume of 1500 L/ha at an average rate of 540 g a.i./ha. Imidacloprid was applied foliarly or as a drench at 148–168 g a.i./ha. Exposure was conducted in compartments that measured 58 m^2 partitioned within the greenhouse. Treatments took place the afternoon and evening before exposure of the bumblebees. One bee colony was placed in each compartment. Hives were opened during the afternoon the day after application to allow bees to forage. Mortality was determined after a 1-wk exposure. Foraging activity was expressed as proportion of flowers observed with "bite marks" (light to dark brown spots on the pistil)

Table 5. Bee exposure to spinosad under greenhouse conditions.

Test type	Test material	Method/crop	Application rate	Effects description	Reference
Greenhouse residue toxicity to bumblebees	480 g a.i./L SC	Exposure to treated tomato plants in greenhouse	540 g a.i./1500 L/ha	Temporal effects on foraging; slight reduction in brood development	Aldershoff 2000
Greenhouse residue toxicity to bumblebees	250 g a.i./kg WDG	Exposure to treated tomato plants in greenhouse	120 g a.i./1200 L/ha	No effect on foraging; inhibition of larval growth 2 and 4 d after application	Kaneshi 2000a
Greenhouse residue toxicity to honeybees	250 g a.i./L SC	Exposure to treated strawberry plants in greenhouse	100 g a.i./1000 L/ha	Inhibition of larval growth following exposure 1 and 3 d after application	Kaneshi 2000b

over the total number of flowers in each compartment. Flowers were observed 2, 4, and 6 d after initiation of exposure. Colonies were moved to the laboratory for additional observations 7 d after application (DAT). Effects on colony development were assessed by evaluating mortality from 7 through 14 DAT and by counting new adult and immature offspring for the entire period of the test.

After 7 d of exposure, cumulative mortality in the untreated controls was 21%. Mortality in the spinosad treatment was 1% lower than the controls. None of the treatment groups was statistically different from the negative control. Foraging activity (flowers with bite marks) in the untreated control compartments was relatively constant, ranging from 36% on the second day after exposure to 44% on the 6th day of exposure. In compartments in which imidacloprid was applied as a drench, there was an initial increase in foraging activity that gradually decreased to control levels during the course of the experiment. Imidacloprid treatment as a spray had an initial large reduction in foraging activity that increased toward the end of the experiment but did not reach the level of the untreated controls. Spinosad treatment resulted in an initial reduction in foraging, but the activity was similar to the controls at the end of the observation period.

No additional mortality was observed during the time the colonies were maintained in the laboratory. New adult offspring were noted in all treatments, with an average of 7 new workers in each replicate for the water control and 9 in the spinosad treatment. The mean number of immature offspring (pupae and larvae) per untreated control colony was 38.3 ± 14.2, whereas the spinosad treatment group had 22.7 ± 4.2, not statistically significant. Assessment of total brood development indicated an average of 45.3 ± 11.0 individuals for the untreated controls and 31.3 ± 7.2 in the spinosad treatment, again not statistically significant. These data demonstrate that dry residues of spinosad applied at 540 g a.i./ha were not acutely harmful to the bumblebee under greenhouse conditions, although there was a slight numerical reduction of brood.

The toxicity of spinosad (250 g a.i./kg WDG) to foraging bumblebees was also assessed under greenhouse conditions (Kaneshi 2000a). Spinosad was applied to tomatoes at 120 g a.i./ha in a spray volume of 1200 L/ha. Two vinyl greenhouses were employed, one as a control and one for a single spinosad application. Ten colonies, each containing 80–90 adult bees, were used. After application of spinosad, a colony was placed in each greenhouse every 2 d through 8 d after application. Each colony was allowed to forage for 2 d, after which it was removed and held under laboratory conditions. Laboratory observations included the number of adults that returned to the colony and the effect on egg and larval development. Before the introduction of a colony to the greenhouse, egg chambers with mature eggs were opened and the number of eggs counted. After removal from the greenhouse, the condition of the eggs and hatched larvae was followed until adults emerged. Mature larvae (third to fourth instar) were counted based on the shape of the larval chamber. After removal from the greenhouse, these larvae were followed until adults emerged. Foraging activity, identified as bite marks on flowers, was recorded daily from 1 d through 10 d after application.

There was no difference in foraging activity between spinosad-treated and untreated greenhouses for all colonies. When compared to the controls, there was a reduction of the number of adult bees that returned to the colonies that were introduced on days 0 and day 2 to the spinosad-treated greenhouse. There was a 74% and 87% return rate for the spinosad colonies on day 0 and day 2, respectively; similar data for the controls were 97%–98%. There was no difference in return rate between the treatment and controls for colonies introduced at 4, 6, and 8 d after application. There was an apparent effect of spinosad application on egg and early larval development. Inhibition of development was observed in colonies placed in the greenhouse at 0, 2, and 4 d after application (Table 6). Inhibition was not as dramatically reduced in the 6-d colony and was not observed in the colony introduced 8 d after application. There was also inhibition of growth of late-stage larvae in colonies introduced at 0, 2, and 4 d after spinosad application, although an improvement of growth was observed in colonies introduced on 6 and 8 d after application (Table 7). These data demonstrate that spinosad applied in a greenhouse may result in a transient effect on bumblebee development in hives used for greenhouse pollination.

Strawberries The toxicity of spinosad (250 g a.i./kg WDG) residues on strawberries to honeybees was also evaluated (Kaneshi 2000b). Spinosad was applied at 100 g a.i./ha in 1000 L/ha. Four vinyl greenhouses were employed, one for the control, and three replicates of a single spinosad application. After the application of spinosad, each greenhouse was well ventilated to allow the foliage to dry. Four hives, each equipped with a dead bee trap, were held in the control greenhouse and one was introduced into a spinosad-treated greenhouse on 1, 3, and 7 DAT. On the day of application, 200 young (first to second) instar larvae and 4- to 6-d-old larvae (older larvae) were marked. Growth conditions such as delay of eclosion and formation of pupae were recorded every 5 d until all larvae in the control became adults. Behavior of the queen (including ovicidal activity, lethargy, and ataxia) was assessed on 1–10, 15, 20, 25, and 30 DAT. Mortality was recorded on the same schedule as that used for behavior observations. Foraging activity was evaluated by counting the number of foraging bees between 10:00 A.M. and 2:00 P.M. for 10 min on 1–10, 15, and 20 DAT.

No unusual behavior of the queen or workers was observed nor was there any evidence of a reduction of foraging activity or excessive worker mortality. Spinosad affected growth of young larvae that were introduced to the greenhouse 1 and 3 DAT however, eclosion of pupae was unaffected. Pupation of young (first and second instar larvae was 67.5%, 79.0%, and 99.5% for the beehives introduced to the spinosad-treated greenhouse 1, 3, and 7 DAT, respectively (Table 8). Similarly, survival of later-stage larvae was affected by spinosad with pupation of 49.5%, 62.5%, and 99.5%, for the beehives introduced to the spinosad-treated greenhouse at 1, 3, and 7 DAT, respectively (Table 9). Total development was adversely affected as a consequence of retarded pupation. These data demonstrate that spinosad applied in a greenhouse may result

Table 6. Bumblebee early-stage larval development following exposure to spinosad at 120 g a.i./ha.

Day of introduction of colony	Treatment	Number of eggs and young larvae before treatment	Number of larvae recovered	Number of pupae	Percent pupation	Number of adult eclosion	Percent eclosion
0	Spinosad untreated	30	0	0	0	0	0
		30	30	30	100	30	100
2	Spinosad untreated	30	0	0	0	0	0
		30	30	30	100	30	100
4	Spinosad untreated	30	11	3	10	3	10.0
		30	30	29	96.7	29	96.7
6	Spinosad untreated	30	24	20	66.7	20	66.7
		30	30	30	100	30	100
8	Spinosad untreated	30	30	30	100	30	100
		30	30	30	96.7	29	96.7

Table 7. Bumblebee late-stage larval development following exposure to spinosad at 120 g a.i./ha.

Day of introduction of colony	Treatment	Number of middle-stage to late-stage larvae before treatment	Number of pupae	Percent pupation	Number of adult eclosion	Percent eclosion
0	Spinosad untreated	80	3	2.5	0	0
		80	80	100	79	98.8
2	Spinosad untreated	80	2	2.5	0	0
		80	79	98.8	79	98.8
4	Spinosad untreated	80	54	67.5	20	25.0
		80	80	100	80	100
6	Spinosad untreated	80	72	90.0	70	87.5
		80	79	98.8	79	98.8
8	Spinosad untreated	80	80	100	79	98.8
		80	79	98.8	79	98.8

Table 8. Early-stage honeybee larvae development following exposure to strawberries treated with spinosad at 100 g a.i./ha.

Days after treatment for introduction of beehive into greenhouse	Number of larvae assessed	Number of larvae pupated	Pupation (%)	Eclosion of pupae	Eclosion (%)	Total development (%)
	A	B	B/A	C	C/B	C/A
Day 1	200	135	67.5	134	99.3	67.0
Day 3	200	158	79.0	157	99.3	78.5
Day 7	200	199	99.5	199	100	99.5
Untreated	200	199	99.5	199	100	99.5

A, B, and C are in place to facilitate the explanation of the derivation of % pupation, % eclosion and % total development.

in a transient effect on honeybee development in hives used for greenhouse pollination.

Greenhouse studies suggest that there is a potential for effects on hive development when bees are exposed to spinosad under confined conditions. Additional studies may be needed to assess the long-term consequences of spinosad exposure under greenhouse conditions on hive viability.

D. Semifield Studies

Two semifield studies were conducted to evaluate the effect of spinosad under controlled outdoor conditions and represent a higher tier in the hazard characterization process (Table 10).

Table 9. Midstage to old honeybee larvae development following exposure to strawberries treated with spinosad at 100 g a.i./ha.

Days after treatment for introduction of beehive into greenhouse	Number of larvae assessed	Number of larvae pupated	Pupation (%)	Eclosion of pupae	Eclosion (%)	Total development (%)
	A	B	B/A	C	C/B	C/A
Day1	200	109	54.5	99	90.8	49.5
Day 3	200	125	62.5	107	85.6	53.5
Day 7	200	199	99.5	199	100	99.5
Untreated	200	198	99.0	198	99	99.0

Table 10. Semifield studies on bees foraging in spinosad-treated tansy phacelia (*Phacelia tanacetifolia*).

Test type	Test material	Method/crop	Application rate	Effects description	Reference
Toxicity of residues to the honeybee	480 g a.i./L SC	Confined field exposure to treated *Phacelia tanacetifolia*[a]	144 or 540 g a.i./ 1500 L/ha	Temporal effects on foraging; slight reduction in brood development	Vinall 2000
Toxicity of residues to the honeybee	480 g a.i./L SC	Confined field exposure to treated *Phacelia tanacetifolia*[a]	216 g a.i./1500 L/ha	Temporal effects on foraging; no effect on brood development	Halsall 2001

[a]Tansy phacelia.

Tansy Phacelia Vinall (2000) assessed effects of a single application of spinosad (480 g a.i./L SC) at 144 or 540 g a.i./ha in 1500 L/ha on hives of honeybees placed in a flowering crop of tansy phacelia or fiddleneck (*Phacelia tanacetifolia* Benth). Methods were based on EPPO guideline No. 170 (EPPO 1992). Nucleus hives of bees were confined under large cages (4×4.5 m in area) placed over the flowering crop. There were four treatments, each applied in three replicate cages. Two treatments of spinosad, dimethoate applied at 800 g a.i./ha as a toxic reference, and water control were included. Applications were made in the early morning before bees were active in the crop. Assessment of bee mortality in the cages was made during 2 d before treatment and 7 DAT. The number of workers foraging over the crop at a given time, and the number entering or leaving the hive over a fixed period, were also recorded during four observation intervals on days −2, −1, 0, 1, 2, 3, 5, and 7. The condition of the brood and food reserves in the hives was assessed at the start and end of the test.

There was no effect on mortality in the spinosad treatments (Fig. 3). There was no apparent reduction in hive activity, with the numbers of bees moving in and out of the hives in individual treatments remaining relatively stable. Before product application, the daily cycle of foraging activity was similar for all treatments. Following spraying, the numbers of bees foraging in the dimethoate treatment declined dramatically whereas the numbers of foraging bees recorded for

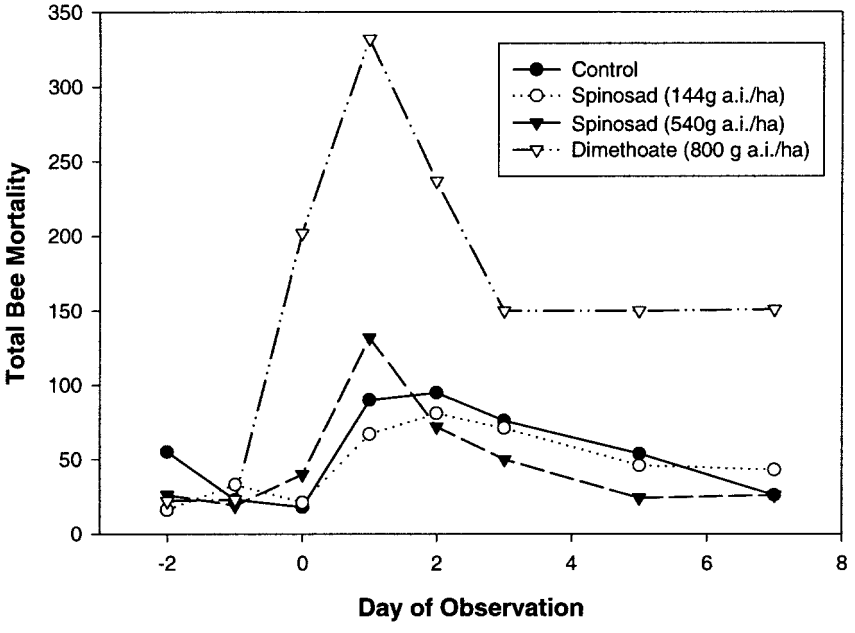

Fig. 3. Daily mortality of honeybees following the application of spinosad and dimethoate to flowering tansy phacelia (*Phacelia tanacetifolia*).

540 g a.i./ha spinosad was reduced relative to the control on the day of application and in most assessments made 2, 3, 5, and 7 DAT. There was a small, but much less marked, decline in levels of foraging activity in the 144 g a.i./ha rate of spinosad. Because there was no marked increase in bee mortality, the observations on the 540 g a.i./ha rate suggested that the product residues might be repellent to the bees and thus discouraged foraging. Posttreatment observations showed that the amount of brood in hives declined in all treatments, including the controls. Mean reduction of brood cells was 33% in the control, 56% at 144 g a.i./ha, 60% at 540 g a.i./ha, and 55% in the toxic reference treatment. Other observations indicated that, in two hives at 540 g a.i./ha, there were some new worker bees that may have died during emergence and some uncapped brood cells with dead bees. These data indicate that an early-morning application of spinosad at a rate of 540 g a.i./ha in 1500 L/ha resulted in a reduction of foraging activity of honeybees, presumptively due to repellency from the recently treated crop. However, there was not a concomitant increase in worker mortality. Minor effects on the survival of brood were detected for two of three hives in this treatment. No significant harmful effects were noted at the 144 g a.i./ha rate. None of the treatments resulted in death of the queen.

A study by Halsall (2001) had a more complex experimental design to assess cumulative effects of repeated application of spinosad to a flowering crop of tansy phacelia. Methods were based on EPPO guideline No. 170 (EPPO 1992). There were four treatments; each applied to three replicate plots in the phacelia crop. Treatment 1 (spinosad 4 × treatment) received four applications of spinosad (480 g a.i./L SC) applied at a rate of 216 g a.i./ha at times T_1, T_2, T_3, and T_4, with successive spray intervals of 0, 7, 17, and 9 d, respectively. Treatment 2 (spinosad 1× treatment) had one application of spinosad at a rate of 216 g a.i./ha on one occasion, at time T_4. Treatment 3, the negative control, received tap water. Treatment 4, the toxic reference treatment, received dimethoate (400 g a.i./L) applied at 400 g a.i./ha at time T_4. The application volume for all treatments was 1500 L/ha. Bees from small nucleus hives (~3000–5000 workers) were confined under cages (4 × 4.5 m) assembled over a flowering crop of tansy phacelia on the evening before the T_4 application. The T_4 application was made at midday when bees were active in the crop. Assessments of bee mortality in the cages were made during the morning and evening for 2 d before the T_4 application and for 5 DAT. Behavior was assessed by recording the number of worker bees foraging over the crop during a 2-min period and by counting the number of bees entering or leaving the hives during a 2-min period. The condition of the brood was evaluated at the start and end of the trial.

Following the T_4 application, before which all hives were placed in the holding cages, the number of dead bees rose most noticeably in the dimethoate treatment group. Mortality in both spinosad treatment regimes did not differ from the controls on any assessment date. The total number of dead bees collected posttreatment was 267, 325, 251, and 454 for treatments 1 (4 × spinosad), 2 (1 × spinosad), 3 (control), and 4 (dimethoate), respectively. Foraging activity between hives was similar to that preceding pesticide application. Following the

T_4 application, the number of bees foraging in the individual treatments only differed significantly ($P < 0.05$) on 4 of 18 observations. Before treatment, healthy queen bees were observed in all the hives. One was not observed, in one of the control replicates, during the posttreatment assessment. There was a consistent decline in brood in all treatments including the control following pesticide application with a mean reduction of occupied brood cells of 44% in the control, 48% in the 1 × spinosad treatment, 51% in the 4 × spinosad treatment, and 42% in the dimethoate treatment. These data indicate that spinosad applied to flowering tansy phacelia at a rate of 216 g a.i./ha did not adversely affect honeybee mortality, foraging activity, or brood development.

E. Field Studies

Field studies provide the most definitive indication of the potential of a pesticide to affect pollinator populations. The effects of spinosad on honey bees exposed under actual use conditions were evaluated in alfalfa, citrus (orange), tree nuts (almonds), kiwifruit, and avocado to provide information on a wide range of crops using different application methods (Table 11).

Alfalfa Spinosad (240 g a.i./L SC) was applied to flowering alfalfa under normal field conditions to four experimental plots (Mayer 1999). It was applied on two plots by helicopter in a volume of 47 L/ha at 70 or 175 g a.i./ha. A positive control (carbaryl) was applied at 1 kg/ha, and there was an untreated control plot. Five colonies of bees were placed in one corner of each plot, and each was screened and covered with a cloth before spraying. After the residues had weathered for approximately 3 hr, the screens and cloth were removed and the bees allowed to forage. Each colony was fitted with a dead bee trap. Mortality was recorded for 5 d before pesticide application and for 5 d following treatment. Foraging activity, the number of honeybees per square meter per 15 sec foraging on the alfalfa flowers (10 replications) was recorded in each plot. Observations were made on the day before treatment, the day of treatment and on 1–5 DAT. The number of frames of brood and the number of adult bees in each colony were again recorded 9 d after treatment.

No effect on morality or foraging activity was observed following the application of spinosad at either 70 or 175 g a.i./ha. In the carbaryl treatment plot, significant mortality occurred on 1 and 2 DAT, and there was a reduction in foraging activity on 1 DAT. Posttreatment total mortality was 721, 833, 813, and 2620 for the control, spinosad 70 g a.i./ha treatment, spinosad 175 g a.i./ha treatment, and carbaryl treatment, respectively. There was no effect on brood development for any treatment group (Table 12).

Almonds Potential effects on adult and immature honeybees was evaluated following the application of spinosad (240 g a.i./L SC) to flowering almond trees (Forey 1999). Two plots were established, one 3.6-ha plot for spinosad application and a 4.5-ha plot for control observations. Spinosad was applied at

Table 11. Field studies on bees foraging in spinosad-treated crops.

Test type	Test material	Method/crop	Application rate	Effects description	Reference
Field exposure of honeybees	240 g a.i./L SC	Field exposure to flowering alfalfa	70 or 175 g a.i./47 L/ha	No effect following 3 hr weathering of residues	Mayer 1999
Field exposure of honeybees	240 g a.i./L SC	Field exposure to flowering almond trees sprayed at night	100 g a.i./941 L/ha	No significant effects noted	Forey 1999
Field exposure of honeybees	240 g a.i./L SC	Field exposure to flowering citrus sprayed at night	157 to 210 g a.i./790 L/ha	No significant effects noted	Kirkland 1999
Field exposure of honeybees	120 g a.i./L SC	Field exposure to flowering kiwifruit	96 and 192 g a.i./ 2000 L/ha	No effect following evening or early morning treatment	Goodwin and Haine 1998
Field exposure of honeybees	120 g a.i./L SC	Field exposure to flowering avocado	96 g a.i./2000 L/ha	No effect following evening treatment	Taylor and Goodwin 2000

Table 12. Summary of brood and adult honeybees (mean number/hive) following field exposure to flowering alfalfa treated with spinosad.

		10 d pretreatment		10 d posttreatment	
Treatment	Rate/ha	Brood	Adults	Brood	Adults
Spinosad	70 g	12.2a[a]	22.7a[a]	12.8a[a]	23.0a[a]
Spinosad	175 g	9.4a	9.4a	10.4a	21.2a
Carbaryl	1 kg	11.2a	11.2a	10.2a	21.6a
Control	—	11.2a	11.2a	11.8a	24.6a

[a]Means within a column followed by the same letter are not significantly different at the $P = 0.05$ level, Newman–Keuls studentized range test.

night at approximately 100 g a.i./ha in 941 L/ha using a Rears Orchard Sprayer. Two days before spraying, five replicate beehives were placed on the edge of each test plot. Each hive was opened before moving the bees to the test site, and a frame containing brood was removed, examined, and marked for identification. The number of uncapped brood was also recorded, and the tray was photographed, and the number of capped cells was counted from the photograph. A dead bee trap was attached to the entrance of each hive. Each hive was covered before spinosad application; the cover was removed immediately following completion of the spray treatment. Dead bee counts began 2 d before application and continued through 12 DAT. Foraging activity was evaluated at 13 hr posttreatment. The number of foraging bees observed in 1 min was recorded for each of 10 randomly chosen trees in each plot. Counts were also taken of the number of flowers visited by a few bees in each plot to ascertain differences in behavior of bees relative to recently sprayed flowers.

The daily and cumulative counts of bees were similar throughout the study, indicating that there was no significant difference in mortality of bees exposed to the trees sprayed with spinosad and the controls. Mortality ranged from 5 to 243 dead bees/trap/d in the spinosad block versus 4–246 bees/trap/d in the untreated block. A total of 2140 dead bees were counted posttreatment in the untreated block versus 2122 dead bees in the spinosad block.

Spinosad application had no effect on posttreatment brood development. The spinosad block averaged 763 occupied cells per frame (larvae, prepupae, and pupae), versus 980 cells per frame in the untreated block. The proportion of capped and uncapped brood cells was also similar (Fig. 4). There was no effect on foraging behavior and, on the basis of flower visits and pollen or nectar collection, there was no evidence that the bees were repelled by spinosad.

Citrus The effect of spinosad on honey bees foraging in flowering orange trees was investigated by Kirkland (1999). Flowering orange trees were sprayed at 157–210 g a.i./ha in 790 L/ha using a Swanson Orchard Sprayer. Two plots

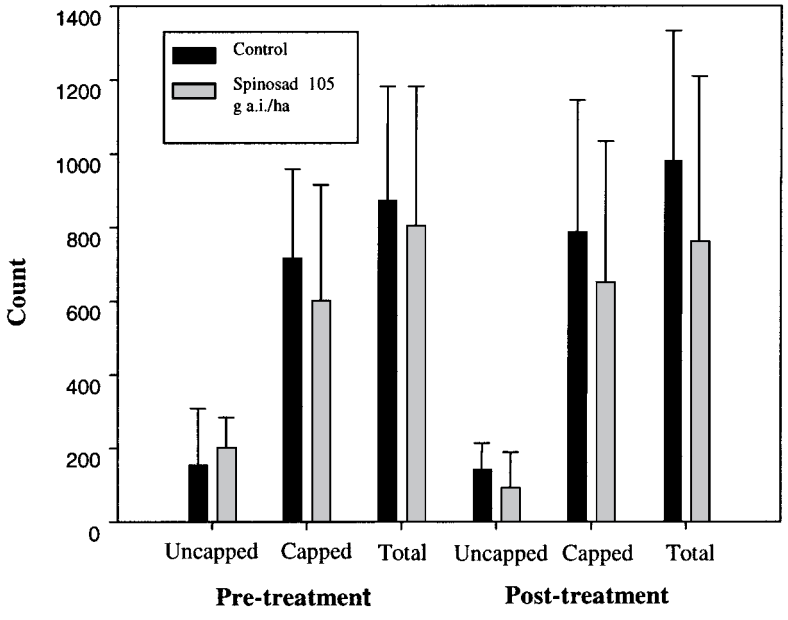

Fig. 4. Brood development of honeybee foraging in almonds treated with spinosad at 105 g a.i./ha.

were established within the orchard, one containing 14 rows (> 50 trees/row/plot) for spinosad application and one containing 24 rows (> 50 trees/row) for the control plot. Twelve hours before spraying, five replicate beehives were placed on the edge of each test plot. Each hive was opened before moving the bees to the test site, and a frame containing brood was removed, examined, and marked for identification. The number of uncapped brood was counted. The tray was also photographed, and the number of capped cells was counted from the photograph. A dead bee trap was attached to the entrance of each hive. Covers were placed on the hives before spinosad application and removed following completion of the spray. Dead bee counts began 12 hr before application and continued through 12 DAT. The number of foraging bees observed in 1 min was recorded for each of six randomly chosen locations in each plot. Counts were also taken of the number of flowers visited by a few bees in each plot to ascertain differences in behavior of bees to recently sprayed flowers.

The number of dead bees recovered from the spinosad-treated block was consistently higher, but no statistical difference ($P = 0.05$) was detected between the control and treated blocks (Fig. 5). A total of 2074 dead bees were counted in the spinosad block versus 1525 dead bees in the untreated. The number of dead bees collected in both blocks was high but within an acceptable range. Mortality was attributed to stress during moving.

Brood development was not affected by spinosad treatment. There was a

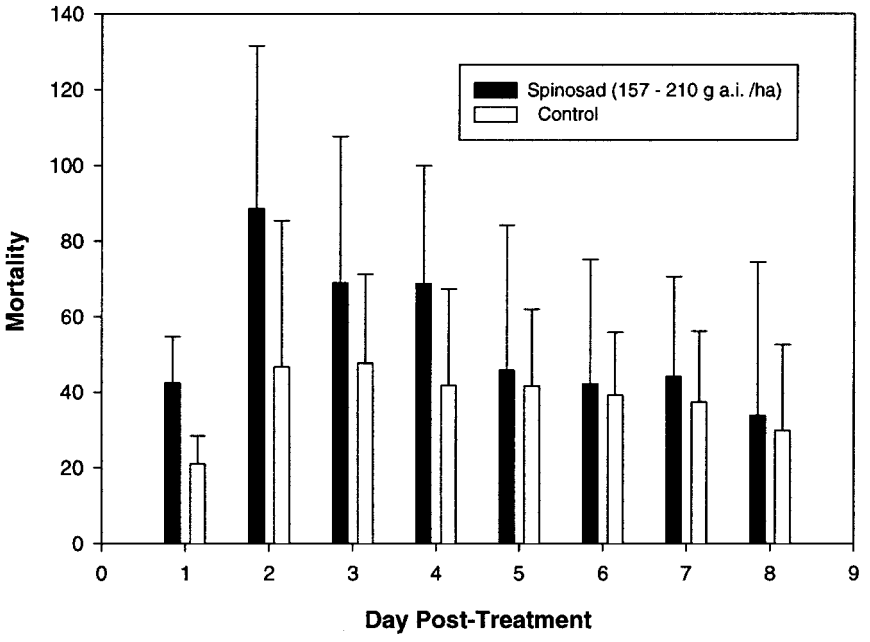

Fig. 5. Mortality of honeybees foraging in citrus following the application of spinosad at 157–210 g a.i./ha.

decrease in occupied cells in both the spinosad treatment and the control hives. A number of capped cells were examined, and all pupae and larvae appeared healthy. Likewise, foraging behavior appeared normal and similar in both blocks, and all worker bees visiting flowers in both blocks appeared healthy and normal.

Burns et al. (2001) conducted a study to assess the efficacy of spinosad bait-spray for the control of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), and the Caribbean fruit fly, *Anastrepha suspensa* (Loew), in citrus orchards. The study also included an assessment of the effect of spinosad on honey bee brood development and hive condition. Spinosad was applied as a tank mix containing a fruit-fly attractant. Four replicate 3.2-ha test plots, each containing approximately 309 trees/ha, were used for the treatment and control. Two honeybee hives were placed in the center of the control and treatment plots 1 wk before treatment. Spinosad was applied as the fly-bait formulation at 0.25 g a.i./ha in a volume of fruit-fly attractant of 21.5 L/ha. Three applications were made at 2-wk intervals. Brood development was assessed by counting the number of frames of brood for each hive and averaging the observations for each block. The condition of the bees was recorded at 24 hr pretreatment and at 14-d intervals. Hive condition was rated by an experienced apiarist on a scale of 1–5 (dead–strong) and recorded as the average of the two hives in each block. No

adverse or measurable effects on brood development or on hive condition were observed following three spinosad treatments over a 6-wk period (Table 13).

Kiwifruit The toxicity of spinosad (120 g a.i./L SC) to honeybees exposed to sprayed kiwifruit, *Actindia deliciosa* (A. Chev.), was evaluated by Goodwin and Haine (1998). Spinosad was applied at 96 g a.i. or 192 g a.i./ha, and tau-fluvalinate, the positive control, was applied at 96 g a.i./ha. Treatments were applied to full coverage using a handheld spray gun at a rate of approximately 2000 L/ha. The vines were 12 yr old and planted on T-bars with a vine spacing of 2.5 m and a row spacing of 5 m. The orchard had a staminate: pistillate vine ratio of 1:6. Spraying was conducted at approximately 40% staminate vine flowering. Separate groups of vines were sprayed in the evening (5:30–7:00 P.M.) and the morning (6:00–7:00 A.M.). Each treatment consisted of 10 randomly selected staminate vines, each of which was separated by another staminate vine and a 4–6 pistillate vine buffer. The staminate vines were spread over three orchard blocks.

Between 19 and 23 foraging bees (identified by the presence of a pollen load) were taken from staminate flowers (from the evening and morning treatment groups) following the morning application. Captured bees were placed in holding cages and provided a 2M-sugar diet. The cages were held in darkness at a temperature of 20°C. Mortality was recorded 24, 48, and 72 hr after capture.

The average bee mortality combining all treatment groups was 1.7% during the first 24 hr of capture; 20.6% had died by 72 hr (Fig. 6). These data demonstrate that the evening or early morning application of spinosad at 96 g a.i. or 192 g a.i./ha to kiwifruit does not affect the survival or foraging of honeybees exposed to pollen or nectar.

Avocado Taylor and Goodwin (2000) evaluated the effect of a single night application of spinosad (120 g a.i./L SC) to avocado trees (*Persea americana* Mill) on honeybee brood development. Of 14 avocado orchards, 7 were treated with spinosad (96 g a.i./ha in 2000 L/ha) and the others served as controls. Four honeybee colonies were randomly placed in the orchards at least 1 d before spinosad treatment. Brood area and brood viability were assessed pre- and post-treatment.

There was no significant difference in brood viability between the treated and control orchards. Brood viability preapplication was 69% and 63% for treated and control orchards, respectively. Postapplication, there was about a 7% decrease in average viability for bees in both the treated and control orchards. The average increase in brood area for the control and treated orchards was 75 cm^2 and 20 cm^2, respectively (Fig. 7). This increase was not statistically different ($P = 0.885$ at 95% confidence). These data indicate that a single application of spinosad at a rate of 96 g a.i./ha at night and allowed to dry before bees begin foraging does not affect brood viability.

Table 13. Honeybee brood number and hive conditions following successive treatments of citrus with spinosad fruit-fly bait (0.28 g a.i./ha).

		24-hr pretreatment		14 d post first treatment		14 d post second treatment		14 d post third treatment	
Treatment	*n*	Brood	Condition	Brood	Condition	Brood	Condition	Brood	Condition
Control	8	6.25 ± 0.56	3.88 ± 0.40	7.00 ± 0.68	3.62 ± 0.42	6.12 ± 0.72	3.75 ± 0.37	5.62 ± 0.86	3.50 ± 0.19
Spinosad/fruit-fly attractant	8	7.25 ± 0.62	4.12 ± 0.40	7.88 ± 0.74	4.25 ± 0.25	7.25 ± 1.08	4.38 ± 0.38	6.75 ± 0.80	3.75 ± 0.31

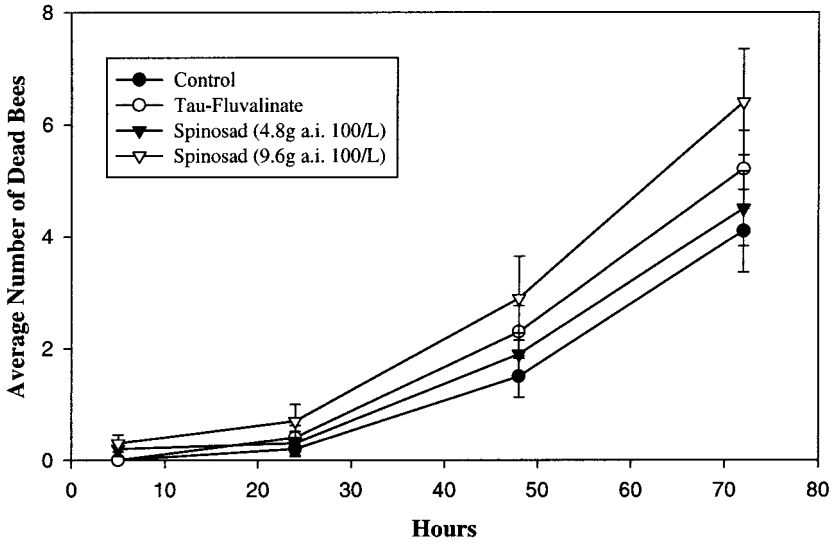

Fig. 6. Honeybee mortality following exposure to kiwifruit flowers treated with spinosad and tau-fluvalinate: morning application.

VII. Discussion

Risk assessment is a process focused on estimating the potential of an adverse advent. Essential to risk analysis is the development of a conceptual framework that describes the potential interaction of the stressor (pesticide in this case) and biological entity of concern. The conceptual framework for this purpose can be stated as follows: pollinators (the entity of concern) can be exposed to spinosad (the stressor) through direct contact during spraying, from residues on treated foliage and flowers, or potentially via nectar and pollen. Once a conceptual framework has been formulated, an assessment of effects and exposure can be conducted; this can be an interative process during which higher levels of complexity in the characterization of effects or exposure or both may be necessary. Evaluation of these data leads to a determination of potential risk and an understanding of risk management opportunities.

Laboratory studies in which technical spinosad was applied directly to the body of bees or provided in the diet have demonstrated that spinosad is intrinsically toxic to bees (see Table 1). Dilute formulations show lesser toxicity. Intuitively, it is evident that compounds that demonstrate high intrinsic toxicity but are managed to limit exposure may not present a high risk to nontarget species. This relationship can be and generally is validated by field data. The data presented here for spinosad exemplifies this relationship.

Research has demonstrated that spinosad residues on alfalfa or on kiwifruit flowers that have been allowed to dry for 3 hr are not acutely harmful to honeybees, as has been demonstrated for low- and ultralow-volume sprays. Further,

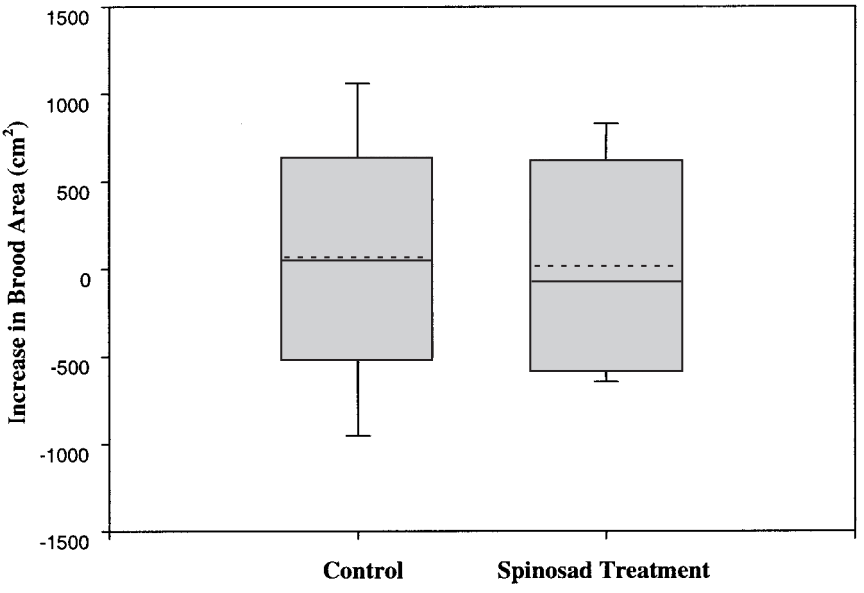

Fig. 7. Brood area (cm^2) pre- and postapplication of spinosad (9.6 g a.i. 100/L) to flowering avocado trees. *Dotted line*: mean; *solid line*: median; *range of the box*: 25th to 75th percentiles; *whiskers*: 5th and 95th percentile.

greenhouse and semifield studies have demonstrated that dried residues are not acutely toxic but that pollen and nectar from sprayed plants may have transient effects on brood development. Field studies on a variety of crops in which applications were made when bees were not active have demonstrated that spinosad has low acute risk to adult honeybees and has little or no effect on hive activity and brood development. The collective evidence from these studies indicates that once spinosad residues have dried on plant foliage (generally 3 hr or less) the hazard of spinosad to a honeybee is negligible.

An initial assessment of the risk of pesticides to pollinators generally is relegated to the use of a hazard characterization scheme. In the U.S., this scheme has been based on the intrinsic toxicity of the product determined in laboratory contact LD_{50} studies with the honeybee to develop label hazard statements (U.S. EPA 1996) (Table 14). Recently, the U.S. EPA has proposed a new policy on Bee Precautionary Labeling (U.S. EPA 2000) and, in some cases, has implemented its use. The proposed policy, among other things, includes statements that reflect "... the length of time in hours or days that the residues of the pesticide remain a toxic threat to bees." Thus, laboratory or field data that provide information on the residual toxicity of residues will be crucial to characterize risk.

Table 14. U.S. EPA honeybee toxicity groups and precautionary statements.

Toxicity group	Precautionary statement if extended residual toxicity is displayed	Precautionary statement if extended residual toxicity is not displayed
I: Product contains any active ingredient with acute LD_{50} of 2 µg/bee or less	This product is highly toxic to bees exposed to direct treatment or residues on blooming crops or weeds. Do not apply this product or allow it to drift to blooming crops or weeds if bees are visiting the treatment area.	This product is highly toxic to bees exposed to direct treatment or residues on blooming crops or weeds. Do not apply this product or allow it to drift to blooming crops or weeds while bees are actively visiting the treatment area.
II: Product contains any active ingredient(s) with acute LD_{50} of greater than 2 µg/bee but less than 11 µg/bee	This product is toxic to bees exposed to direct treatment or residues on blooming crops or weeds. Do not apply this product if bees are visiting the treatment area.	This product is toxic to bees exposed to direct treatment. Do not apply this product while bees are actively visiting the treatment area.
III: All others	No bee caution required.	No bee caution required.

In the United Kingdom, the potential exposure concentration as well as toxicity is included in the hazard assessment (Pesticide Safety Directorate 1996). Thus, in the U.K. a hazard ratio (i.e., application rate in g a.i./ha ÷ LD_{50} µg a.i./bee) is calculated, and if the quotient is less than 50 the product is not labeled. If the quotient is greater than 2500, the product must be labeled as "High Risk to Bees do not apply to crops in flower or those in which bees are actively foraging. Do not apply when flowering weeds are present." If laboratory or field data describing conditions under which mortality, foraging activity, and behavior are not affected are provided to the regulatory authority, this label statement can be removed.

Mayer et al. (2001) have also addressed residue toxicity to bees. They evaluated the toxicity of spinosad residues on alfalfa resulting from different application rates to the honeybee, alkali bee, and leafcutter bee. Using the residual degradation time in hours required to reduce bee mortality to less than 25% (RT_{25}), they found that for rates up to 0.2 kg a.i./ha the RT_{25} was less than 2 hr for honeybees. For alkali bees and leafcutter bees, the RT_{25} was less than 2 hr for application rates of 0.05 and 0.1 kg a.i./ha, respectively, and greater than 2 hr but less than 8 hr for 0.2 kg a.i./ha.

The development of best agricultural practices based on a RT_{25} for bees has been proposed. Johansen and Mayer (1990) suggested that pesticides with a RT_{50} of 2 hr or less may be applied ether during the early morning, late evening,

or at night to flowering crops where bees are foraging. Pesticides with a RT_{50} of greater than 2 hr but less than 8 hr should only be applied in the late evening.

This collective evidence indicates that spinosad residues, once dried, are not acutely toxic to adult honeybees and also that under field conditions dried residues do not overtly affect bee behavior or brood development. Application practices that limit the direct exposure of honeybees to the spray and that allow at least 3 hr drying of the residues will greatly reduce the risk of spinosad to honeybees. Good agricultural practices should include timing high-rate applications to when bees are not actively foraging or notifying beekeepers of spray schedules and/or closing hives before spraying to ensure that spinosad will not impact bees.

The U.S. EPA has embraced this concept for spinosad. For bees, the label environmental hazard statement for concentrated SC formulations of spinosad states "This product is toxic to bees exposed to treatment for 3 hr following treatment. Do not apply this pesticide to blooming, pollen-shedding or nectar-producing parts of plants if bees may forage on the plants during this time period. The 3 hr limitation does not apply if the applicator operates in a state with a formal, state-approved bee protection program and the applicator follows all applicable requirements of the state-approved program designated to ensure that managed bees are not present in the treatment area during this time period." The U.S. EPA required no bee hazard statement for the dilute fruit-fly bait formulation.

Summary

Spinosad is a natural insecticide derived from an actinomycete bacterium species, *Saccharopolyspora spinosa* (Mertz and Yao 1990), that displays the efficacy of a synthetic insecticide. It consists of the two most active metabolites, designated spinosyn A and D. Both spinosyns are readily degraded in moist aerobic soil, and field dissipation, which is quite rapid (half-life, 0.3–0.5 d) can be attributed to photolysis or a combination of metabolism and photolysis.

Spinosad causes neurological effects in insects that are consistent with the general activation of nicotinic acetylcholine receptors but by a mechanism that is novel among known insecticide compounds. Spinosad has a high level of efficacy for lepidopteran larvae, as well as some Diptera, Coleoptera, Thysanoptera, and Hymenoptera, but has limited to no activity to other insects and exhibits low toxicity to mammals and other wildlife.

Although spinosad has low toxicity to most beneficial insects, initial acute laboratory tests indicated that spinosad is intrinsically toxic to pollinators. The hazard of spinosad to bees was evaluated using a tiered approach. Initial acute laboratory exposures were conducted, followed by toxicity of residues of spinosad on treated foliage, greenhouse studies to assess acute as well as chronic toxicity, confined field assessments, and finally full field studies using a variety of crops under typical use conditions.

These data were used to assess the potential of adverse effects on foraging

bees following the use of spinosad. This research has clearly demonstrated that spinosad residues that have been allowed to dry for 3 hr are not acutely harmful to honeybees when low-volume and ultralow-volume sprays are used. Further, glasshouse and semifield studies have demonstrated that dried residues are not acutely toxic, and although pollen and nectar from sprayed plants may have transient effects on brood development, the residues do not overtly affect hive viability of either the honeybee or the bumblebee. Field studies in which typical application methods of spinosad were used on a variety of crops have demonstrated that spinosad has low risk to adult honeybees and has little or no effect on hive activity and brood development. The collective evidence from these studies indicates that once spinosad residues have dried on plant foliage, generally 3 hr or less, the risk of spinosad to honeybees is negligible.

Appendix: Identification of Other Insecticides Mentioned in the Text

Common name	Chemical name	Class	CAS registration number
Carbaryl	1-naphthyl methylcarbamate	Carbamate	63-25-2
Dimethoate	*O*,*O*-Dimethyl *S*-methylcarbamoylmethyl phosphorodithioate	Organophosphorus	60-51-5
Imidacloprid	1-(6-Chloro-3-pyridymethyl)-*N*-nitroimidazolidin-2-ylideneamine	Nicotinoid	105827-78-9
Tau-fluvalinate	(*RS*)-α-Cyano-3-phenoxybenzyl *N*-(2-chloro-α,α,α-trifluoro-*p*-tolyl)-D-valinate	Pyrethroid	102851-06-9

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Manuscript received August 2; accepted December 12, 2002.

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Rev Environ Contam Toxicol 179:73–97

Polycyclic Aromatic Hydrocarbon Ecotoxicity Data for Developing Soil Quality Criteria

John Jensen and Line E. Sverdrup

Contents

I. Introduction

Soil quality standards, soil quality criteria, or soil screening levels are considered valuable tools for assessing the environmental risk of contamination. The objective of ecotoxicological soil quality criteria (SQC) is to give guidance on concentration limits for various chemicals to protect the function and structure of ecosystems. Because of the generic and universal nature of these criteria, they are not suitable for site-specific assessment of environmental risk. For an assessment of actual risk, adequate data for the effects of a compound should ideally be obtained from the ecosystem of interest. That is, effects of a certain compound on the structure as well as the function of the ecosystem alone and in combination with other compounds should be investigated. However, it is

Communicated by G.W. Ware.

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obvious that all organisms and functions in an ecosystem cannot practically be tested against all possible compounds and combinations of these, and this is why risk assessors accept using the more approximate screening tools. In most cases, the SQC are based on data from a few single-species laboratory tests, and by applying "assessment factors," "safety factors," or "uncertainty factors," these data are used for an extrapolation to effects of the chemical on ecosystems.

Polycyclic aromatic hydrocarbons (PAHs) are common soil pollutants. Land may be contaminated with PAHs through, for example, oil and gas spills, atmospheric deposition, sewage sludge application, or pollution from old gas works or sites used for drying tar-coated fishing nets. The widespread contamination of soils by PAHs has created a need for simple tools for a first screening of risk. Ecotoxicological SQC for PAHs already exist in a number of countries (e.g., Denmark, The Netherlands, and Canada), and they are widely used despite the fact that there has been an obvious lack of compatible toxicity data. The existing SQC for PAHs are calculated from a small number of relevant terrestrial ecotoxicity data, or even derived on the basis of aquatic toxicity data. When aquatic data are used, toxicity must be recalculated into soil exposure data by using the equilibrium partitioning theory, which is described in such sources as the Technical Guidance Document (TGD), which is the guideline for assessing risk of industrial chemicals in the European Union (European Commission 1996). Obviously, the use of aquatic data is not ideal for assessing terrestrial toxicity, and the large number of useful ecotoxicity data recently generated within a large Danish/Norwegian research program therefore is a significant step forward in establishing sound SQC for PAHs. In the project, the chronic toxicity of four PAHs and four N-, S-, O-heterocyclic aromatic compounds was determined by using three soil invertebrates [springtails (*Folsomia fimetaria*), earthworms (*Eisenia veneta*), and enchytraeids (*Enchytraeus crypticus*)], and three plant species [mustard (*Sinapis alba*), red clover (*Trifolium pratense*), and ryegrass (*Lolium perenne*)]. In addition, soil microbial toxicity was evaluated through the effects on nitrification. The results of this investigation showed that springtails appeared to be the most sensitive of the species tested (Sverdrup 2001), and an additional group of 12 PAHs was therefore tested with springtails only. These new toxicity data, found in Tables 1 and 2, have made it possible to recalculate the existing ecotoxicological soil quality criteria for PAHs.

II. Review of Terrestrial Ecotoxicity Data

Ecotoxicity data for the group of PAHs have been collected. A large amount of such ecotoxicity data was recently generated in our laboratory (Sverdrup 2001, Sverdrup et al. 2001, Sverdrup et al 2002a–d). The data were generated using a well-described, agricultural soil with a relatively low organic carbon content (1.6%), which makes the values relatively conservative in terms of exposure conditions. In all the studies, standard procedures were used and exposure concentrations were measured. The use of the same soil type and very similar exposure conditions (see original papers for details) made these data optimal for

Table 1. Measured effect concentrations for four polycyclic aromatic hydrocarbons (PAHs) affecting springtails, enchytraeids, earthworms, microorganisms and plants.

	Endpoint	Effect level	Pyrene	Fluoranthene	Phenanthrene	Fluorene
Springtails[a]	Reproduction	EC_{10}	10	37	23	7.7
Enchytraeids[b]	Reproduction	EC_{10}	11	15	40	25
Earthworms[c]	Growth	EC_{10}	30	90	20	25
Nitrification[d]	Rate	EC_{10}	130	13	42	33
Mustard[e]	Growth	EC_{20}	403	595	171	969
Ryegrass[e]	Growth	EC_{20}	>1000	240	152	176
Red clover[e]	Growth	EC_{20}	28	58	23	38

All data in mg kg^{-1} soil dry weight.
[a]Sverdrup et al. (2001).
[b]Sverdrup et al. (2002a).
[c]Sverdrup et al. (2002b).
[d]Sverdrup et al. (2000c).
[e]Sverdrup (2001).

comparing species sensitivity (i.e., without the usual interlaboratory uncertainties related to differences in soil types, animal cultures, etc.). These data are presented in Tables 1 and 2. Supplementary toxicity data are available in the open literature. However, many of these are not directly applicable for the derivation of soil quality standards according to the algorithm used in this review, either because the organisms were not exposed through a soil medium or because the organisms were exposed through spiked or historically contaminated soils polluted with a mixture of contaminants. In other cases, no useful toxicity

Table 2. Toxicity of various PAHs to the springtail *Folsomia fimetaria*.

	LC_{50}, mg kg^{-1}	EC_{10}, mg kg^{-1}
Napthalene	167	20
Acenaphthylene	145	23
Acenapthene	107	31
Anthracene	67	5
Benz[*a*]anthracene	>975	>975
Chrysene	>1025	>1025
Benzo[*b*]fluoranthene	>360	>360
Benzo[*k*]fluoranthene	>560	>560
Perylene	>560	>560
Benzo[*a*]pyrene	>840	>840
Indeno[1,2,3-*cd*]pyrene	>913	>913
Dibenz[*a*,*h*]anthracene	>775	>775

Source: Sverdrup et al. (2002d).

measures were given; e.g., typically no effects were observed at the highest test concentration, or only EC_{50} or LC_{50} values were given.

Finally, several studies have focused on uptake processes and hence exposed the animals or plants to PAHs at concentrations below toxic levels. Although perhaps not directly applicable for the purpose of calculating SQC, these data may be useful in the lines of evidence used in the final judgment. For the benefit of risk assessors, using other selection criteria than those used by the authors of this paper, some of the most relevant studies are briefly reviewed here. Although relatively comprehensive, this review should not be regarded as an all-inclusive review of terrestrial ecotoxicological data for PAHs.

A. Microorganisms

In a study where a total of 100 mg kg^{-1} of anthracene, pyrene, and phenanthrene had been added to two different soils, Lee and Banks (1993) found that microbial abundance was not significantly changed in any of the treatments after 24 wk. Mahmood and Rao (1993) did not observe any decrease in microbial or fungal abundance during 15 mon of exposure to anthracene, phenanthrene, chrysene, pyrene, or fluoranthene with initial concentrations of 1000 mg kg^{-1}. When exposed to anthracene and pyrene, the microbial counts increased significantly, and the fungal numbers increased significantly in all the spiked soils, except in those spiked with chrysene. Hund and Traunspurger (1994) measured nitrification and the microbial respiration rate in a bioremediation test of a natural PAH-contaminated soil. In this soil, 16 PAHs were measured. Initial ΣPAH concentration was 4500 mg kg^{-1}, of which the main part was PAHs with three or four rings. Inhibition of the nitrification processes was observed until the 10th mon of remediation. At this time, the total PAH concentration was reduced to approximately 1750 mg kg^{-1}. No degradation of the PAHs with five or six rings was observed, whereas in the group with three and four rings most of the degradable compounds disappeared, leaving less than 200 and 800 mg kg^{-1}, respectively. As a response to the reduction of the easily degradable carbon source of PAHs with three or four rings, the respiration rate decreased significantly after 10 mon. Eschenbach et al. (1991) studied the effects of 1, 5, and 10 mg kg^{-1} of benzo[*a*]pyrene on the respiration and dehydrogenase activity of the soil, but observed no effects in any of the treatments after 1–10 d. Similarly, Park et al. (1990) found no change in the bacterial or fungal communities in two different soils after more than 100 d of exposure to benzo[*a*]pyrene with a starting concentration of 33 mg kg^{-1}.

B. Plants

Hulzebos et al. (1993) tested the toxicity of 76 organic pollutants, including acenaphthene and naphthalene, to lettuce (*Lactuca sativa*). After 2 wk of exposure, they found an EC_{50} value for acenaphthene of 25 mg kg^{-1}. The EC_{50} value for naphthalene was higher than the highest tested concentration, 100 mg kg^{-1}. Henner et al. (1999) incubated lupine (*Lupinus albus*) seeds in soils spiked with

PAHs in concentrations ranging from 31 to 155 mg kg^{-1}. They saw no inhibition by benzo[*a*]anthracene, benzo[*a*]pyrene, or dibenz[*a*,*h*]anthracene on either germination or growth after 1 mon in the greenhouse. This result supports the lack of toxicity of the high molecular weight PAHs. Maliszewska-Kordybach and Smreczak (2000) evaluated the ecotoxicological effects of soils artifically spiked with a mixture of four PAHs (fluorene, anthracene, pyrene, and chrysene). They exposed six plant species (wheat, oat, maize, tomato, bean, and sunflower) in three different soil types. Concentrations below 10 mg kg^{-1} stimulated rather than inhibited the growth of the plants. The lowest concentration significantly inhibiting (EC_{20}) plant growth was 20 mg kg^{-1} (tomato in sandy soil), whereas the other EC_{20} values exceeded 100 mg kg^{-1}. Phytotoxicity was negatively correlated to the organic matter content of the test soil, indicating a reduced bioavailability in soils rich in organic matter.

Williams and Wiegert (1971) showed, in a 1-yr field study, that repeated application (7–10 d interval during summer and monthly intervals during winter) of 8–10 g naphthalene m^{-2}, corresponding to approximately 175 mg kg^{-1}, had a pronounced effect on the vegetation. All plants were killed within the first 30–60 d after first application, and very few seedlings survived more than 2 mon. Ten months after the last application, only a few seeds had germinated in the treated plots. Using the OECD Test Guideline, Mitchell et al. (1988) followed the effects of anthracene on three crop species and three native Australian plant species. They found that anthracene affected seed emergence and growth with EC_{50} values in the range from 30 to more than 1000 mg kg^{-1} with *Avena sativa* as the most sensitive species. Weber et al. (1984) saw no growth effects of naphthalene, anthracene, benz[*a*]anthracene, or phenanthrene on corn, fescue, or soybean when studied at loading rates of 0.1, 1.0, and 10 mg kg^{-1}, respectively. Environment Canada (CCME 1997) exposed radish (*Raphanus sativa*) and lettuce (*Lactuca sativa*) to benzo[*a*]pyrene and naphthalene in the artificial OECD soil. They observed no effects of benzo[*a*]pyrene on radish at exposure concentration up to 23,800 mg kg^{-1} dry weight, whereas the emergence of lettuce seedlings was significantly inhibited at 11,900 mg kg^{-1}. The lowest test concentration at which they found effects of naphthalene on radish and lettuce was 61 and 3 mg kg^{-1}, respectively. However, they had unresolved problems with recovering all the naphthalene added, and the results should therefore be used with caution.

Wagner et al. (1969) reported a lack of phytotoxic effects of benzo[*a*]pyrene and benzfluoranthene at concentrations up to 1.5 mg kg^{-1} for summer wheat and maize and up to 3.3 mg kg^{-1} for rye seedlings. Similarly, Dörr (1970) reported a lack of benzo[*a*]pyrene toxicity to rye when grown in soil with a concentration of 3.3 mg kg^{-1}. El-Fouly (1980) showed a growth-stimulating effect when cultivating wheat, maize, and beans in quartz sand containing 0.005–0.250 mg kg^{-1} of benzo[*a*]pyrene. The highest stimulation was found at concentrations between 0.01 and 0.05 mg kg^{-1}, and no inhibition of seedling growth was observed up to 2.0 mg kg^{-1}.

Baud-Grasset et al. (1993) constructed an experiment in which they tested

the phytotoxicity of a PAH-contaminated soil from a waste site. A seed germination test showed that the contaminated soil was highly toxic to lettuce, millet, and oat seeds. Germination of the seeds was completely inhibited in soil where the sum of three- and four-ringed PAHs was measured to 5878 mg kg^{-1}. When this soil was mixed with an artificial uncontaminated soil down to a content of 12.5% of the original level of contamination (i.e., 735 mg kg^{-1}), the estimated LC_{50} value for seed germination was 10.1%, 19.9%, and 30.9% of contaminated soil for lettuce, millet, and oat, respectively. This result corresponds to approximate PAH concentrations of 590 mg kg^{-1}, 1170 mg kg^{-1}, and 1815 mg kg^{-1}. In 1994, Hund and Traunspurger reported on the growth of *Brassica rapa* and *Avena sativa* in a bioremediation test of a naturally PAH-contaminated soil. Of the two species, *B. rapa* was most affected by the PAH-contaminated soil. A complete growth inhibition existed through the first 4 mon, and after 11 mon the growth of *B. rapa* was still reduced by 35% as compared to uncontaminated soil. The growth reduction of *A. sativa* was approximately 70%, 50%, and 10% after 0, 4, and 11 mon of bioremediation. After 11 mon of remediation the total PAH concentration was determined at approximately 1750 mg kg^{-1}. Henner et al. (1999) found profound phytotoxicity to seven plant species when exposed to medium (Σ16 PAHs = 1584 mg kg^{-1}) and highly (Σ16 PAHs = 3251 mg kg^{-1}) polluted gas works soils. After 60 d of exposure the growth of plants was reduced by 16%–82% in the medium polluted soil and 70%–100% in the highly polluted soil. Alfalfa (*Medicago sativa*) and red clover (*Trifolium pratense*) were the most sensitive species.

C. Invertebrates

Environment Canada exposed the earthworm *Eisenia fetida* to benzo[*a*]pyrene and naphthalene in the artificial OECD soil (CCME 1997). They observed no adverse effects of benzo[*a*]pyrene at exposure concentrations up to 48,000 mg kg^{-1}, but found a 25% reduction in earthworm survival at a naphthalene concentration of 54 mg kg^{-1}. However, they had unresolved problems with recovering all the added naphthalene, and the authors therefore noted that the results should be used with caution. Neuhauser et al. (1985) tested the impact of fluorene on *E. fetida* in a 14-d artificial soil test and reported an LC_{50} of 173 mg kg^{-1}. A later paper by Neuhauser et al. (1986) presented the results from acute toxicity studies with fluorene on three other earthworm species, i.e., *Allolobophora tuberculata, Eudrillus eugeniae*, and *Perinyx excacatus.* Here, they reported LC_{50} values of 206, 197, and 170 mg kg^{-1}, respectively. In a subsequent paper, Neuhauser and Callahan (1990) determined the sublethal effects of fluorene on *E. fetida*, but the dose–response relationship was not very clear: at 50 and 25 mg kg^{-1} exposure levels, there were no effects. At 100 mg kg^{-1}, a significant ($P < 0.05$) and large (50%) reduction in cocoon production was observed. However, although the worms produced 40% less cocoons than in the control at 150 mg kg^{-1}, the data were not significantly different ($P > 0.05$). Growth was unaffected at 150 mg kg^{-1}, and at 200 mg kg^{-1} all worms died, leaving 150 and 200 mg

kg^{-1} as NOEC and LOEC values for growth. vanBrummelen et al. (1996) cites Bowmer et al. (1993) for a study testing the effects of phenanthrene to the springtail *Folsomia candida* and the earthworm *E. fetida*. According to this citation, Bowmer et al. found a 50% reduction in springtail reproduction at approximately 116 mg kg^{-1} and an LC_{50} of 135 mg kg^{-1} after 4 wk of exposure. The NOEC and LOEC values for reproduction were 70 and 93 mg kg^{-1}, respectively, whereas the lowest concentration not significantly affecting survival was 125 mg kg^{-1}. The 3-wk test with earthworms showed an estimated 50% reduction in reproduction at 225mg kg^{-1} with NOEC and LOEC values of 93 and 300 mg kg^{-1}, respectively. Crouau et al. (1999) found a 50% reduction in reproduction at 175 mg kg^{-1} when exposing *F. candida* to phenanthrene. An LOEC of 220 mg kg^{-1} and a NOEC of 140 mg kg^{-1} were observed for effects on reproduction, and at 380 mg kg^{-1} most adults died.

Römbke et al. (1994) tested the effects of anthracene on various soil invertebrates exposed in the artificial OECD soil. For the earthworm *E. andrei.*, they found an LC_{50} value higher than 1000 mg kg^{-1}, but at concentrations of 200 mg kg^{-1} and above, a small (10%–20%), nonsignificant reduction in the biomass could be detected. For the carabid *Poecilus cupress*, they observed a 15% reduction in feeding rate when exposed to 10 mg anthracene kg^{-1} dry soil, but no mortality or other changes in behaviour were observed. Neither survival nor reproduction of the Collembola *F. candida* seemed to be sensitive to anthracene because both the LC_{50} and EC_{50} values were greater than 1000 mg kg^{-1} (Römbke et al. 1994). In a biomarker study, Eason et al. (1999) exposed the earthworm *Eisenia andrei* to 20 and 100 mg benzo[*a*]pyrene kg^{-1}. They observed no significant effect on mortality, growth, or behavior over the 4-wk exposure. Neutral red retention time, however, was significantly affected at both doses. Joner et al. (2001) found no acute toxicity when exposing *Eisenia fetida* for 2 wk in soil spiked with a mixture of 500 anthracene kg^{-1}, 500 mg chrysene kg^{-1}, and 50 mg dibenz[*a,h*]anthracene. They did, however, observe a small effect (17% mortality) when the worms were exposed for 42 d. The also found that mycorrhizal inoculation had a positive effect on earthworm survival. Achazi et al. (1995) used enchytraeids (*Enchytraeus crypticus*) and earthworms (*Eisenia fetida*) to investigate the ecotoxicity of fluoranthene and benzo[*a*]pyrene. The test parameters were survival rate, growth, cocoon production, and hatching rate. Surprisingly, they found higher toxicity of benzo[*a*]pyrene than of fluoranthene, despite the far higher solubility of the latter. Fluoranthene affected enchytraeid reproduction at soil concentrations above 1200 mg kg^{-1}, whereas 25% reduction in reproduction was observed at 10 mg benzo[*a*]pyrene kg^{-1}.

The same high toxicity was also observed in spiked soil aged 1 and 3 mon before exposure, whereas in soils aged for 5 mon, the 25% effective concentration rose to 100 mg kg^{-1}. Earthworm survival was reduced by nearly 50% at 10 mg kg^{-1} benzo[*a*]pyrene and 55% at 100 mg kg^{-1}, whereas reproduction was reduced to only 10% and 5% of the control at soil concentrations of 10 and 100 mg kg^{-1}, respectively. Growth of adults was stimulated at both concentrations. Because there was a very large discrepancy between the results of Achazi et al.

(1995) and the results of other terrestrial invertebrate toxicity studies with benzo[*a*]pyrene (CCME 1997; Eason et al. 1999; Sverdrup et al. 2002d), the study of Achazi et al. (1995) might therefore benefit from being repeated.

Van Straalen and Verweij (1991) collected the woodlouse *Porcellio scaber* from a wood stack and observed various response parameters in the laboratory when the animals were exposed to benzo[*a*]pyrene in their food. When given food ad libitum containing 1, 5, 25, and 125 mg benzo[*a*]pyrene kg^{-1}, the woodlice showed no change in food consumption or CO_2 production. A sex-dependent response was observed for food assimilation and growth efficiency, however, as only male isopods significantly increased their food assimilation and reduced their growth efficiency when exposed to 125 mg kg^{-1} in food. Females also had lower growth efficiencies at the highest exposure concentration, but due to a high standard variation this was not statistically significant. Van Brummelen et al. (1991) found a linear correlation between benzo[*a*]pyrene assimilation and exposure, but no differences between sexes. Van Brummelen and Stuijfzand (1993) found small growth effects on the two terrestrial isopods *Onicus asellus* and *P. scaber* at 100 mg benzo[*a*]pyrene kg^{-1} food (NOEC = 31.6 mg kg^{-1}), and survival for *O. asellus* was affected when exposed to 316 mg kg^{-1} in food. Van Brummelen et al. (1996) examined the toxicity of five PAHs to the isopod *O. asellus*. When exposed through the food, phenanthrene (706 mg kg^{-1}), fluoranthene (802 mg kg^{-1}), and benzo[*a*]pyrene (316 mg kg^{-1}) had no effect on growth, whereas fluorene and benz[*a*]anthracene caused a small, but significant, growth reduction at 208 and 28.6 mg kg^{-1} (NOEC values of 66 and 9 mg kg^{-1}), respectively.

In a study by Hund and Traunspurger (1994), no individuals of the earthworm *E. fetida* survived when introduced to a naturally contaminated soil for 14 d. The soil contained a total of Σ16 PAHs at 4500 mg kg^{-1}, of which the main part were PAHs with three or four rings. However, after 7 mon remediation, most of the PAHs with three and four rings were degraded, with a significant reduction in toxicity. Even though the total PAH concentration of the soil was still as high as 2000 mg kg^{-1}, all the tested earthworms now survived a 2-wk exposure. Williams and Wiegert (1971) showed, in a 1-yr field study, that repeated application (7–10 d intervals in summer and monthly intervals in winter) of 8–10 g naphthalene m^{-2}, corresponding to approximately 175 mg kg^{-1}, significantly ($P < 0.05$) reduced the number of soil/litter arthropods. Reduction averaged 90% in the treated plots. This reduction occurred in all groups of arthropods, although mites apparently were the most sensitive group.

Erstfeld and Snow-Ashbrook (1999) studied the effects of chronic low levels of PAHs on soil invertebrate communities. Nematode community structure, total abundance of microarthropods (Collembola and Acarina), and biomass of earthworms were evaluated in six sample plots representing a gradient of PAH ranging from 5.28 to 80.46 mg kg^{-1} total PAH. The organic carbon content varied from 3.3% to approximately 40% and pH values ranged from 6.7 to 8.6. They found that abundance of omnivore/predator nematodes and collombolans, the nematode diversity, and earthworm biomass all exhibited positive correlations

with PAH concentrations. Although only small changes were observed, total mite abundance was found to be negatively associated with PAH concentrations. Charrois et al. (2001) investigated the acute toxicity of three creosote-contaminated soils to the earthworm *Eisenia fetida*. The total Σ16 PAH concentration in the two most polluted soils was 1320 and 1500 mg kg^{-1}. The soils appeared to be acutely toxic to earthworms, showing 100% mortality within the first 2 d. Furthermore, even when exposed to soil containing as little as 3% of the contaminated soils, i.e., less than 50 mg PAH kg^{-1}, mortality was 100% after 4 d. This result indicates that other organic constituents in the contaminated soil may have contributed to the observed toxicity. The authors suggested that the ratio between PAH concentration and total (dichlormethane) extractable organics (PAH:DEO) would be a better predictor of toxicity than total PAH alone.

Recently, Blakely et al. (2002) analyzed soil invertebrate and microbial communities in 30 intact soil cores collected from a 50-yr-old creosote-contaminated site. Total abundance of nematodes, Collembola, mites, and total bacterial and fungal biomass were quantified. PAH concentration ranged from 5 to >35,000 mg kg^{-1}. Based on the results, they suggested that nematodes were affected directly by PAH, whereas collembolans and mites primarily were affected through food web changes, e.g., fungi and bacteria, and decomposition-mediated alterations than by direct toxicity. Eijsackers et al. (2001) observed no significant effects on survival and reproduction of earthworms (*Eisenia fetida*) when exposed to sediment containing 2.4 mg PAH kg^{-1}.

III. Calculation of Soil Quality Criteria (SQC) for PAHs

A. Methodologies

Typically, soil quality standards/criteria are derived either by the use of simple "safety" or "assessment" factors or by the use of a statistical extrapolation method using the species sensitivity distribution (SSD) (Posthuma et al. 2002). In the factorial application method, here named the TGD method, a predicted no-affect concentration (PNEC) is calculated by dividing the lowest effect or no-effect level with an "assessment" factor between 10 and 1000. The size of the factor depends on the quality and quantity of available ecotoxicity data (Table 3), as well as on information on the mode of toxic action. The PNEC is defined as the concentration below which unacceptable effects most likely will not occur (European Commission 1996). The exact use of safety factors may vary slightly but is relatively similar between nations and regions; it ranges from the use of a factor of 1000 in cases where only acute toxicity data exist for one or two species to the use of a factor of 10 in cases where chronic toxicity data exist for a number of species covering several trophic levels. The application factor may be less than 10 in cases where sufficient additional information from field surveys and mesocosm studies exist. The aim of the factors involved is, among others, to include intraspecific and interspecific differences, differences between chronic and acute effects, and differences between the effective concentrations found in field and laboratory studies. For a discussion about the use of

Table 3. Indicative assessment factors as summarized in the Technical Guidance Document (TGD) in support of Commission Directive 93/67/EEC on risk assessment for new notified substances and Commission Regulation (EC) No. 1488/94 on risk assessment for existing substances.

Information available	Assessment factor
L(E)C_{50} short-term toxicity tests, e.g., plants, earthworms, or microorganisms	1000
NOEC for one long-term toxicity test	100
NOEC for additional long-term toxicity tests of two trophic levels	50
NOEC for additional long-term toxicity tests of three trophic levels	10
Field data/data of model ecosystems	Case-by-case

safety factors and other problems in deriving SQC, please consult van Straalen (1993), Chapman et al. (1998), and Duke and Taggart (2000).

In cases where adequate data are available, it is possible to use statistical methods that make use of the whole data set rather than only the lowest value. These relatively novel methods are based on the assumption that the sensitivity of all species in an ecosystem can be described by the frequency distribution of the ecotoxicity data that are available. The methods may include only simple frequency distributions of all collected data, from which a certain percentile is chosen as threshold concentration (CCME 1996), or statistical extrapolation methods using species sensitivity distributions. The species sensitivity distribution may be assumed to be log-logistic (Aldenberg and Sloob 1993) or log-normal (Wagner and Løkke 1991), or may be fitted by bootstrapping. In all cases, the distribution is used to derive a protection level for a certain fraction of the soil organisms, e.g., 95%, of all species with a predefined statistical confidence level of, for example, 95% or 50%; i.e., the estimate may not be conservative enough in 5% or 50% of the occasions, respectively. The estimated value is called the HC5 (Aldenberg and Sloob 1993) or the Kp_5 (Wagner and Løkke 1991).

The choice of confidence level for the 95% protection estimate varies between countries (e.g., 95% in Denmark and 50% in the Netherlands), and so does the minimum requirement of toxicity data. Generally, a protection of 95% of all species is considered valid for a full protection of the ecosystem, and hence a multifunctional use of land (Emans et al. 1993; Okkerman et al. 1993). Lower levels of protection may, however, be chosen in cases where a fitness-for-use approach is taken. In such cases, criteria changes according to the land use, e.g., pristine land, parks, or residential or industrial areas. Canada, for example, has two sets of criteria, one for agricultural and residential/park land use (the 25th percentile of all the collected "no-effect" data, i.e., the NOECs or extrapolated NOECs) and another for commercial and industrial land use (25th percentile of all the collected "effect" data, i.e., the LOECs or the EC_{50}s).

The values calculated by the two methods should only be considered as predictions of a negligible or a no-effect concentration and should by no means be considered as "safe" concentrations (European Commission 1996). The soil quality criterion may be proposed on basis of the lower outcome of the two methods, or on only one of them depending on the prescription in the national algorithm. Some countries use the TGD method if the number of NOEC data is less than or equal to a fixed number, e.g., three, and the SSD method when more data are available, whereas others always use both methods if sufficient data are available. Experts and authorities should propose the soil quality criteria after a final evaluation in which information about the chemical fate and background level is taken into consideration. Finally, the soil quality criteria (SQC) should reflect the accuracy of the calculations and the underlying uncertainties; e.g., 11.58 mg kg^{-1} would create a misleading signal of accuracy.

When using the SSD method, a number of assumptions must be tested or accepted. First, it is important to choose the right distribution for the data. For example, if the interspecies variation in sensitivity is described by a log-normal function, this assumption must be tested using, for example, the Kolmogorov–Smirnov one-sample test for a standard normal distribution. Furthermore, the method assumes that the toxicity data used represent the sensitivity range of the species in the ecosystem. For this reason, it has been stressed that various taxonomic groups should be included in the data set to construct a representative set of test organisms. On the other hand, large taxonomic distances should be avoided (Løkke 1994), especially when dealing with specifically acting substances. In cases of specifically acting substances such as herbicides or insecticides, it is hence very important not to mix toxicity data for various groups of organisms in the same sensitivity distribution, but rather perform separate SSD calculations for separate groups of organisms, e.g., plants and invertebrates, individually. In the case of PAHs, however, the literature gives only little indication that PAHs act by a specific mode of action. One exception is the carcinogenic and mutagenic mode of action of some of the heavier PAHs such as benzo[*a*]pyrene. Sverdrup et al. (2002d) suggested that anthracene may have some specific mode of action on terrestrial species as they found a higher toxicity than could be expected on the basis of its chemical characteristics (lipophilicity), as has also been observed for aquatic species (Kalf et al. 1997). However, no other examples of a specific mode of action have been found, and it is therefore assumed that PAHs act primarily by a narcotic mode of action, i.e., a physical disruption of biological membranes.

The estimate of the HC5 depends on three characteristics of the ecotoxicity data used: the average of all data, their standard deviation, and the number of observations. With fixed values of the two others, a higher average will lead to a higher estimate of the HC5. A larger standard deviation will lead to a lower HC5, and an increase in number of test species will lead to a higher HC5. In other words, due to the nature of the extrapolation method a very *low toxicity* to a few species, i.e., higher standard deviation, may lead to lower estimated soil quality criteria. The average of the distribution curve is moved to the right

(i.e., higher concentrations). However, the expanding of the right tail of the distribution will lead to a similar expanding of the left side of the distribution curve, where the HC5 is determined, and hence a lower PNEC or quality criterion.

Finally, the assumption that protection of a certain fraction of the species will protect the function and structure of the entire ecosystem has to be accepted. This assumption has been, and still is, an issue of debate, as questioned by for example Forbes and Forbes (1993), Smith and Cairns (1993) and Forbes and Calow (2002), who argued that structure and function of an ecosystem are often uncoupled, and thus toxicity data for species cannot be used to predict safe levels for ecosystems. At present, however, little knowledge is available to support or disprove such statements. Okkerman et al. (1993), Emans et al. (1993), and Versteeg et al. (1999) compared NOECs derived from multispecies experiments with extrapolated values and data from single-species tests, and concluded that single-species data are a good starting point for the establishment of safe values for aquatic ecosystems. A similar comparison between multispecies experiments and extrapolated values has not been found for the terrestrial environment.

B. Algorithm for Deriving SQC

As mentioned, a number of approaches or algorithms may be chosen when deriving SQC, and these differ widely among nationalities and regions (Fergusson et al. 1998). The collection of data presented in this study will facilitate a potential reevaluation process using any national algorithm. However, in this review the following approach is taken.

1. Both the assessment factor application (TGD method) and the species sensitivity distribution (SSD) methods are considered. For the TGD method, the assessment factors recommended by the EU (European Commission 1996) (see Table 3) are used for calculation of soil quality criteria. For the SSD method, the log-normal distribution is used to describe the sensitivity distribution (Wagner and Løkke 1991), and the estimated lower 95% confidence limit for the 95% protection level is used.
2. In the selection of ecotoxicity data, it was decided to use toxicity data recently generated in the laboratory of the Danish National Environmental Research Institute. Suitable data (statistically derived NOEC or EC_{10} values) for additional species were not found in other studies. We made no attempt to extrapolate "NOEC" values from other effect measures such as EC_{50} or LC_{50} values. Some countries may, however, accept an extrapolation of "no-effect" levels from acute effect levels by the application of safety factors typically ranging from 3 to 10 (Crommetuijn et al. 2000; Kalf et al. 1997). We also neglected a few studies for reasons of inconsistency in the reported data. Finally, we made no attempt to convert aquatic or food experiments into "soil data" by various extrapolations.
3. The SQC are not derived for a "standard soil" but are considered widely applicable for all types of soils, although some consideration about bioavail-

ability and aging processes are made in the discussion and most certainly also should be considered by the risk assessor.
4. We have not considered the potential genotoxic and mutagenic impact of PAHs on soil organisms.
5. Finally, the presented SQC for PAHs do not cover the high molecular PAHs. The arguments for this decision are fully presented next. The criterion is predominantly based on the fact that the bulk of information available covers the group of low molecular PAHs, and that the low water solubility of the high molecular PAHs makes them less bioavailable and hence less toxic for soil-dwelling species.

C. Calculation of SQC for Individual PAHs

TGD Method According to the TGD method, there is sufficient information available to justify the use of an assessment factor of 10 (see Table 3). The lowest "no-effect level" among the most intensively studied PAHs was 7.7 mg kg^{-1} dry weight, which was the level where the springtail *Folsomia fimetaria* reduced reproduction by 10% when exposed to fluorene (see Table 1). Approximately the same EC_{10} level (10 mg kg^{-1}) was found for pyrene, where, again, springtails were the most sensitive species. The corresponding NOEC values for fluorene and pyrene were 13 and 14 mg kg^{-1}, respectively. Based on data in Table 1, the algorithm prescribes PNEC's for individual PAHs in the range of 0.8–2.3 mg PAH kg^{-1} dry soil (Table 4).

Species Sensitivity Distribution (SSD) Method In this review, the log-normal distribution was selected to describe the sensitivity distribution, and the result may therefore differ slightly as compared to risk assessors using a log-logistic distribution. The results of the SSD method are found in Table 4. The calculations with SSD are from one to more than six times lower than the results

Table 4. Predicted environmental no effect concentrations (mg kg^{-1} dry weight) for four PAHs as calculated by the TGD method and the SSD method.

Method	Pyrene	Fluoranthene	Phenanthrene	Fluorene
TGD				
EC_{10} values	10	13	23	7.7
AF	10	10	10	10
PNEC	1.0	1,3	2,3	0.8
SSD				
HC5 ($n = 7$)	0.15	0.56	2.2	0.24

TGD: factorial application method; AF: assessment factor; PNEC: predicted no-effect concentration; SSD: species sensitivity distribution.
Data used for the calculations are found in Table 1.

obtained by the TGD method. The largest difference was found for pyrene and the smallest for phenanthrene. The very low estimate by the SDD for pyrene (0.15 mg kg^{-1}) is partly due to the very low sensitivity of ryegrass to pyrene, which led to a large standard variation between ecotoxicity data and hence lower HC5 values. If removing the ryegrass data, which is not a real EC_{20} value as the effect was lower than 20% at the highest exposure concentration, the HC5 for the remaining six data is 1.9 mg kg^{-1}.

TGD Method Versus SSD Method The previous sections have presented two sets of PNEC calculations for deriving SQC for PAHs. In general, the output from the two methods did not vary significantly. There may be pros and cons for both methods, but it is beyond the scope of this review to discuss the relative applicability of the various distributions. For an exhaustive description of SSD methods, readers are referred to Posthuma et al. (2002). However, comparisons have shown that for relatively large data sets the difference is insignificant compared to other uncertainties connected to the risk assessment procedures for contaminated soils (Løkke 1994). Roman et al. (1999) discussed the robustness and preciseness, i.e., dispersion of results, for three different extrapolation methods. They concluded that for small sample sizes the application factor method (TGD method) was more, or just as, stable and precise as the SSD method, especially compared to the SSD calculations using 95% confidence limits. By randomly sampling subdata sets ($n = 3–11$) among a large data set ($n = 11$), they concluded that all methods, on average, had a conservative level of protection (expressed as percent protected species from the large data set) covering a range of 95%–99.9% of all tested species. By using toxicity data for eight substances, they found that PNEC values calculated by the TGD method always were lower than the PNEC calculated by the SSD using the 50% confidence limit (SSD-50). However, compared to the SSD calculations using the lower 95% confidence limit (SSD-95), the TGD method only once calculated a lower PNEC and twice calculated similar PNEC values. Finally, Roman et al. concluded that the critical sample size where the results from the TGD method became more disperse than the SSD-50 and the SSD-95 was approximately six and eight species, respectively. The present data set for PAHs contains three invertebrate species, three plant species, and one microbial process, all in all seven data. The present calculations with soil quality criteria for PAH are hence an intermediate situation according to the conclusions made by Roman et al. (1999).

D. Calculation of Sum Criteria for PAHs

The previous section has presented the calculation of SQC for individual PAHs. It is, however, unlikely that organisms will be exposed to one single PAH alone because PAH contamination almost exclusively exists as a mixture of PAHs and related compounds. Although it is easy to administrate only individual criteria, it would be relevant to calculate SQC for the sum of a predefined number of PAHs, which then also needs to be determined chemically. As it is unrealistic

to expect chemical and toxicological data to be available on all possible PAHs, it is recommended to decide on a realistic and appropriate number of relevant PAHs for a sum criterion. This approach is currently the case in many countries, e.g., the six Borneff PAHs (fluoranthene, benzo[*a*]pyrene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*ghi*]perylene, and indeno[1,2,3,-*cd*]pyrene), the 10 VROM PAHs (napthalene, phenanthrene, anthracene, fluoranthene, chysene, benz[*a*]anthracene, benzo[*a*]pyrene, benzo[*k*]fluoranthene, benzo[*ghi*]perylene, and indeno[1,2,3,-*cd*]pyrene) or the 16 USEPA PAHs (napthalene, acenaphthene, acenaphthylene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, chrysene, benz[*a*]anthracene, benzo[*a*]pyrene, benzo[*k*]fluoranthene, dibenz[*a,h*]anthracene, benzo[*b*]fluoranthene, indeno[1,2,3,-*cd*]pyrene, and benzo[*ghi*]perylene).

We suggest deriving *ecotoxicological* soil quality criteria, which focus on the lower molecular PAHs, i.e., those with log K_{ow} values lower than 5.5 or 6. In cases where soil-dwelling organisms (primarily) are exposed through pore water, this is the log K_{ow} range where a cutoff in toxicity is expected for narcotic substances (Sverdrup et al. 2002d). To reduce costs in assessing ecological risk, we recommend including only six to eight low molecular PAHs in a sum criterion. As an example, we have chosen the following eight PAHs as representatives for the relatively low molecular PAHs: acenaphthene (154, 3.95), fluorene (166, 4.2), anthracene (178, 4.5), phenanthrene (178, 4.6), pyrene (202, 4.9), fluoranthene (202, 5.2), benz[*a*]anthracene (228, 5.7), and chrysene (228, 5.8), with molecular weight and log K_{ow} values in parentheses. Currently, there exist only a relatively comprehensive data set for four of these PAHs (see Table 1). For other PAHs, relevant toxicity data are only available for springtails (see Table 2). On the other hand, the four substances intensely investigated showed that springtails, on average, are the most sensitive of the organisms tested (Table 1) (Sverdrup 2001); this could justify the derivation of SQC based on the springtail data only.

If applying a high degree of safety, e.g., by using the most toxic substance as a representative of the entire group of PAHs, the SQC for the sum of eight PAHs could hence be recommended at 1.0 mg kg^{-1} or even 0.5 mg kg^{-1}. This usage is, nevertheless, a relatively conservative approach, applied on an already conservative methodology. Another pragmatic, but not necessarily scientifically sound approach, would be to choose the average toxicity toward the investigated PAHs. Springtails seem on average to be the most sensitive species, with a "mean EC_{10}" of approximately 19 mg kg^{-1} when including pyrene, fluoranthene, phenanthrene, fluorene, acenapthene, and anthracene, but this increases to more than 260 mg kg^{-1} if including benz[*a*]anthracene and chrysene. The use of "mean toxicity" is, however, only scientifically valid if the exposure concentrations of all compounds are similar, and the effects are additive and dose–response curves parallel, which would almost never be the case.

Instead, a hazard index or toxicity equivalency factor approach may be used (Safe 1998). In the toxicity equivalency factor approach it is assumed that the compounds in a mixture act by the same biological or toxic pathway, that the

effects are additive, and that the dose–response curves are parallel. We have no reason to believe this should not, in rough terms, be the case for PAHs. The toxicity equivalent (TEQ) is calculated by multiplying the concentration of each compound (C_i) in a mixture by the relative toxic potency of the individual compounds in the mixture to an index compound (TEF_i), i.e., $\mathrm{TEQ} = \Sigma\ C_i \times TEF_i$. The resulting TEQ is assumed to be an equivalent dose of the index compound and can hence be compared to the PNEC or SQC for the index compound. If the TEQ is greater than the SQC, the mixture may constitute a risk.

It is strongly encouraged to use the toxicity data presented in this study in the TEQ approach when assessing the risk at a specific site. Pyrene could in that case be a valid index compound. However, it is also in more general terms possible to refine a generic sum criterion for PAHs by including information about the relative abundance of PAHs. Although some variation in the composition of PAHs is found between sites, a typical distribution of individual PAHs in aged soil samples may be forecasted. Table 5 contains information about PAH concentrations in a number of Danish soil samples collected from kindergardens, garden allotments, and old sites used for drying fishing nets after tar coating. These three different types of sites represent different sources of pollution: soils from kindergardens are typically contaminated by diffuse air pollution, and so are the allotments, but the latter ones may also have been placed on old dumping sites. The drying places are representative for contamination with tar components also found at old gas works. The observed abundance of individual PAHs may or may not be useful for other countries, and national information may be used instead.

By using "typical" relative abundance instead of exact concentrations in the TEQ approach, the generic SQC for a "typical" mixture may be calculated:

$$\mathrm{TEQ} = \Sigma\ RA_i \times TEF_i$$

and hence

$$\mathrm{SQC_{mix}} = \mathrm{TEQ} \times SQC_{index}$$

where *RA* is the relative abundance of the individual PAH compared to the total concentration of PAHs in the mixture, e.g., 6, 8, or 16 (here we used the average values for the three pollution categories found in Table 5)

TEF is the relative toxicity of the PAH compared to the index compound (here expressed as EC_{10} values from the springtail tests)

SQC_{mix} is the sum criterion for the mixture of PAHs

SQC_{index} is the criterion for the index compound (here, pyrene)

If only the index compound is present, the SQC correspond to the EC_{10} value of that compound divided by an assessment factor of 10. If all substances are found at equal levels, the SQC for the mixture correspond to the average EC_{10} value divided by the safety factor of 10. By using the Danish data in Table 5,

Table 5. PAH concentrations in Danish soil samples collected from numerous soils among three different types of sites: data for benzo[*b*]fluoranthene and benzo[*k*]fluoranthene are summed.

	Kindergardens $n = 288$	Garden allotment $n = 380$	Drying sites $n = 282$	Kindergardens $n = 288$	Garden allotment $n = 380$	Drying sites $n = 282$
	mg kg^{-1} dry wt.			%		
Napthalene	0.15	—	0.68	0.8	—	0.9
Acenaphthylene	0.27	—	1	1.4	—	1.3
Acenapthene	0.09	—	0.29	0.5	—	0.4
Fluorene	0,19	—	0.68	1.1	—	0.9
Phenanthrene	1.45	2.79	5.5	7.7	11.1	7.3
Anthracene	0.4	—	1.5	2.2	—	2.0
Fluoranthene	2.59	5.44	12	13.7	21.6	16.0
Pyrene	7.0	4.61	11	37.1	18.3	14.7
Benz[*a*]anthracene	1.02	2.38	6.4	5.4	9.5	8.5
Chrysene	1.13	2.55	6.2	6.0	10.1	8.3
Benzo[*b* + *k*]fluoranthene	1.96	4.61	11	10.4	18.3	14.7
Benzo[*a*]pyrene	1.1	2.8	6.1	5.8	11.1	8.1
Indeno[1,2,3-*cd*]pyrene	0.76	—	6.2	4.1	—	8.3
Dibenz[*a*,*h*]anthracene	0.14	—	1.2	0.7	—	1.6
Benzo[*g*,*h*,*i*]perylene	0.63	—	5.3	3.4	—	7.1
Sum PAH	18.88	25.18	75.05	100	100	100

Source: Knudsen et al. (2001).

this method leads to a SQC of 1.3 mg kg^{-1} for the sum of the six PAHs (acenaphthene, fluorene, anthracene, phenanthrene, pyrene, and fluoranthene). The same calculations covering eight substances, i.e., including benz[*a*]anthracene and chrysene, gives a sum criterion of 25 mg kg^{-1}. The difference of more than an order in magnitude is due to inclusion of two additional compounds in the sum criterion that typically are present in relatively high concentration but are far less toxic than the remaining six. Using the same approach for the USEPA 16 PAHs, the ecotoxicological sum criterion could be estimated to be more than 35 mg kg^{-1}. This criterion is, however, associated to large uncertainties because very few exact EC_{10} or NOEC values are available for the high molecular PAHs, as they typically did not cause any adverse effects even at the highest test concentration (see Table 2). It is important to stress that all these calculations are indicative and only applicable in cases that do not differ significantly from the outlined Danish situation.

Knowing the concentrations of all the individual PAHs makes it possible to estimate site-specific numbers. The average sum-concentrations of acenaphthene, fluorene, anthracene, phenanthrene, pyrene, and fluoranthene in 288 samples from Danish kindergardens, allotments, and drying sites (see Table 5) averaged approximately 12, 13, and 31 mg kg^{-1}, respectively, which is at least 10 times higher than the calculated sum criterion of 1.3 mg kg^{-1}. The average level of acenaphthene, fluorene, anthracene, phenanthrene, pyrene, fluoranthene, benz[*a*]anthracene, and chrysene were approximately 14, 18, and 44 mg kg^{-1}, respectively, which only in the case of the highly contaminated drying sites were higher than the calculated sum criterion of 25 mg kg^{-1}. By including the Σ16 USEPA PAHs, the total concentrations averaged approximately 19, 25, and 75 mg kg^{-1} for kindergardens, allotments, and drying sites, respectively, compared to the calculated sum criterion for this group of PAHs of more than 35 mg kg^{-1}. Again, only the tar-contaminated sites, used for drying of fishing nets, have average concentrations above the criteria.

E. Final Verdict for SQC

It is quite obvious from the foregoing calculations of PNEC values and SQC that the result depends strongly on the selection of substances included in the sum criteria, as well as other decisions made by the risk assessor. For example, which methodologies should be used, the TGD or the SSD, or both? Which safety factor should be used in the TGD or which level of certainty should be used in the SSD, e.g., the 50% or the 95% confidence limit? Should the procedure include the use of "no-effect levels" extrapolated from the result of acute tests with mortality as endpoint? Should the SQC be derived for individual substances, or should it be derived for the sum of a well-defined number of substances? In that case, which substances should be included in the sum criteria? Should the SQC be based on the worst case principle, or should it be based on some kind of toxicity equivalence? Should there be only one set of

"multifunctional" SQC, or is a land use-dependent SQC more relevant? The last criteria would reflect different demands on soil quality according to the fitness-for-use approach (van de Leemkule et al. 1999; Van Hestereen et al. 1999).

From these questions, it is evident that very different SQCs may be derived depending on the assumption and algorithm used. We have presented the available documentation and have made our calculations so transparent that any risk assessor should be able to apply their own algorithm on the data and hence achieve results suitable for their situation. If generic values are needed, we suggest the following individual criteria: pyrene, 1.0 mg kg^{-1}; fluorene, 1.0 mg kg^{-1}; fluroanthene, 1.5 mg kg^{-1}; phenanthrene, 2.5 mg kg^{-1}; and the following sum criteria: sum of acenaphthene, fluorene, anthracene, phenanthrene, pyrene, fluoranthene, benz[*a*]anthracene, and chrysene, 25 mg kg^{-1}.

IV. Applicability and Aspects of Uncertainty

No matter the algorithm used and no matter the criteria derived, there are a number of things that the risk assessor must keep in mind. Before using any statistically derived quality standards for practical applications, it is important to recognize their inherent limitations and associated uncertainty. There are many elements of uncertainty associated with the extrapolation of effects observed in studies using single chemicals spiked under well-controlled and optimal conditions, to the inhomogeneous and suboptimal situation dominating at most field sites. Exposing animals or plants to historically contaminated soils often reveals a low toxicity even though measured concentrations typically exceed any existing quality criteria. The low toxicity of aged hydrophobic compounds is often ascribed to a significant reduction in bioavailability over time (Alexander 2000; Sverdrup et al. 2002e). Furthermore, bioavailability and hence toxicity differ between soils because of differences in soil properties such as the quantity and quality of organic matter (Chung and Alexander 2002).

Because of these circumstances it is very difficult, if not impossible, to extrapolate the results obtained in one freshly spiked soil to multiple soil types aged for various years. It is therefore the objective of many research projects (e.g., www.liberation.dk) to investigate the importance of bioavailability when assessing ecological risk and the possibility of using chemical or biological tools for assessing the bioavailable fraction of PAH contamination. These research activities, it is hoped, may facilitate and increase the reliability of ecological risk assessment of contaminated soil and sediments in the future. Until then, it must be strongly emphasized to use only soil quality criteria, as with other environmental standards, as a screening tool for assessing ecological risk. These criteria should never alone lead to remediation or other costly actions. Other site-specific tools such as bioassays (i.e., testing the toxicity of contaminated field soils) or field surveys of ecosystem structures at the population or community level are recommended as supplementary higher-tier tools for the risk assessor.

V. Conclusions

Predicted no-effect concentrations (PNEC values) and soil quality criteria (SQC) for soil dwelling species were calculated using various assumptions and two internationally accepted methods, i.e., application of assessment factors and the species sensitivity distributions, respectively. Predicted values from the two methods were similar, and the soil quality criteria vary mostly as a result of the assumptions made by the risk assessor, e.g., whether to have a sum criterion and, if so, which chemicals to include in such a criterion. Based on existing data, we believe that when assessing the risk for ecological effects on soil-dwelling organisms it would be most useful to focus on PAHs with log K_{ow} below 6.0, as toxicity is typically absent for PAHs with log K_{ow} higher than 5.5–6.0

Calculations showed that for four individual PAHs with three or four rings SQC fall in the range of 1.0 and 2.5 mg kg^{-1}. However, as no individual PAH is found alone it may be useful to use a sum criterion for a group of PAHs. Based on the toxicity data presented here, and the average abundant of different PAHs in nearly 1000 Danish soil samples, an ecotoxicological soil quality criterion of 25 mg kg^{-1} dry weight for the sum of the eight PAHs acenaphthene, fluorene, anthracene, phenanthrene, pyrene, fluoranthene, benz[*a*]anthracene, and chysene is suggested.

There are many elements of uncertainty associated with the extrapolation of SQC. It must therefore strongly be emphasized that soil quality criteria should only be used as a screening tool for assessing ecological risk. Other site-specific tools such as bioassays or field surveys of ecosystem structures at the population or community level are recommended as supplementary higher-tier tools for the risk assessor.

Summary

With the overall perspective of calculating soil quality criteria (SQC) for the group of polycyclic aromatic hydrocarbons (PAHs), the existing ecotoxicity data for the soil compartment have been reviewed. The majority of data useful in the context of deriving SQC are of recent origin. Soil quality criteria are considered valuable tools for assessing the environmental risk of contamination, as they may give guidance on concentration limits for various chemicals to protect the function and structure of ecosystems. Soil quality criteria for soil-dwelling species were calculated using various assumptions and two internationally accepted methods, i.e., application of assessment factors and species sensitivity distributions, respectively. It was suggested to derive ecotoxicological soil quality criteria, which focus on the lower molecular weight PAHs, i.e., those with log K_{ow} values lower than 5.5 or 6; this is the log K_{ow} range where a cutoff in toxicity for terrestrial species is expected for narcotic substances. Predicted values from the two methods were similar. Calculations showed that, for four individual PAHs of three or four rings, SQC fall in the range of 1.0 and 2.5 mg kg^{-1}.

However, as no individual PAH is fond alone it is suggested to use a sum criterion for a group of PAHs instead. The different possibilities to calculate such a sum criterion are discussed. Based on toxicity data presented here and the average abundance of different PAHs in nearly 1000 Danish soil samples, an ecotoxicological soil quality criterion of 25 mg kg^{-1} dry weight for the sum of the eight PAHs acenaphthene, fluorene, anthracene, phenanthrene, pyrene, fluoranthene, benz[*a*]anthracene, and chrysene is suggested.

Acknowledgment

Financial support from the Centre for Biological Processes in Contaminated Soil and Sediment (BIOPRO) under the Danish Environmental Research Programme, and the European Research Project "Development of a Decision Support System for Sustainable Management of Contaminated Land by Linking Bioavailability, Ecological Risk and Ground Water Pollution of Organic Pollutants" (LIBERATION) (contract no. EVK1-CT-2001-00105) is acknowledged.

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Manuscript received December 4; Accepted December 17, 2002

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Rev Environ Contam Toxicol 179:99–121

Environmental and Toxicity Effects of Perfluoroalkylated Substances

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Contents

I. Introduction

Various publications on the occurrence of perfluorinated chemicals in the natural as well as the work environment (Gilliland and Mandel 1993; Key et al. 1997; Giesy and Kannan 2001; Kissa 2001) recently have raised scientific and political interest in these compounds. Most of the studies on perfluorinated compounds have focused on perfluorooctyl sulfonate (PFOS) and perfluorooctanoic acid (PFOA). These two chemicals (Fig. 1) are the most important degradation products of the perfluoroalkylsulfonates, which form, together with the perfluoroalkylethylates, the vast majority of the perfluoroalkylated substances (PFAS).

Communicated by Pim de Voogt.

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Fig. 1. Chemical structures of perfluorooctyl sulfonate (PFOS) and perfluorooctanoic acid (PFOA).

Some 30 naturally occurring organofluorines are known to exist. Most biogenic fluorinated chemicals contain only one fluorine atom per molecule (Key et al. 1997; Gribble 2002). The polyfluorinated chemicals are produced because of their specific physical and chemical properties, chemical and thermal inertness, low surface energy, and special surface-active properties (Smart 1994; Kissa 2001). These characteristics have led to a wide variety in PFAS applications, ranging from carpet protection to fire-fighting foams. PFAS are used around the globe in industrialized and urbanized areas. Although the distribution and biodegradation of PFAS are not completely understood, their detection in biota worldwide, including remote locations such as the Arctic (Giesy and Kannan 2001), has caused international scientific and societal attention (Browne 2000; Clarke 2001; USEPA 2000; Renner 2001).

The main manufacturer of perfluoroalkylsulfonates, the 3M company, has performed various studies on the environmental fate and effects, toxicology, and pharmacokinetics of these substances. The results of these studies have been published mainly in the "gray," or non-peer-reviewed, literature. Recently, joint perfluoroalkylethylates manufacturers have started a large research effort covering the same topics for their products (APME 2002; TRP 2002).

In this chapter, the state of knowledge about the application and routes of emission of PFAS is reviewed, followed by a discussion of their behavior in the environment, their occurrence, and their toxicology. As an example, an overview of the use of PFAS for various applications in the Netherlands is presented, which can be considered relevant for the European Union, because similar applications can be found throughout the member states.

Many of the data presented here have been retrieved from the gray literature and from personal communication with several researchers, representatives of industry, and users of PFAS. Therefore, some of the references are difficult to obtain and some statements do not have clear, unequivocal references. The present review is based on an extensive literature study (Hekster et al. 2002) that is available from the authors upon request.

II. Production

A. Electrochemical Fluorination

In 1944, the electrochemical fluorination (ECF) process was developed by Simons et al. 3M has used this route of production since 1956 to produce perfluoroalkylsulfonates (3M 1950; Noel et al. 1996). In the ECF process, the organic

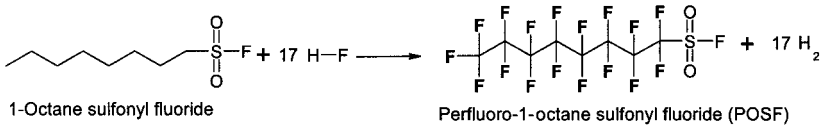


Fig. 2. Example of the electrochemical fluorination (ECF) process.

compound is dissolved or dispersed in anhydrous hydrogen fluoride. A direct electric current is passed through the hydrogen fluoride, causing all the hydrogen atoms on the organic compound to be replaced by fluorine. The overall reaction is shown in Fig. 2.

Perfluoro-1-octane sulfonylfluoride (POSF) is the starting product for the range of products based on perfluorooctylsulfonates (or C8-perfluoroalkylsulfonates). This compound is made to react with methyl or ethylamine, and subsequently with ethylene carbonate to form *N*-methyl (*N*-MeFOSE) or *N*-ethylperfluorooctanesulfonamidoethanol (*N*-EtFOSE). These two compounds are the primary building blocks for the perfluorochemistry of 3M (3M 2000a). The ECF process is an impure process. The reaction shown in Fig. 2 leads to several by-products, as presented in Table 1 (3M 1999, 2000a, 2001a).

In 2000 the 3M corporation decided to phase out the perfluorooctyl chemistry. This decision was based on "[. . .] principles of responsible environmental management" (3M 2000b). For some applications the production of PFAS is being continued (USEPA 2002a), but otherwise it is believed that 3M will replace the perfluorooctyl chemistry with the butyl equivalent.

B. Telomerization

The perfluoroalkylethylates are produced via telomerization. This process was developed by Haszeldine in 1949 and adapted by DuPont in the 1969s (DuPont 1964; Rao and Baker 1994). This process is used by, among others, AsahiGlass, AtoFina, Clariant, Daikin, and DuPont. In the first stage of this process, perflu-

Table 1. Impurities in the electrochemical production of perfluoro-1-octane sulfonylfluoride (POSF).

Percentage	Substance
35%–40%	*n*-POSF
20%–25%	Perfluorinated alkanes and ethers
18%–20%	Branched non-C8 perfluorinated sulfonyl fluorides
10%–15%	Tars (high molecular weight fluorochemical by-products) and molecular hydrogen
7%	Linear non C8-perfluorinated sulfonates

Source: 3M (1999, 2000a, 2001a).

1) $5\,C_2F_4 + IF_5\ (\text{from } I_2 + F_2) + 2I_2 \xrightarrow{catalyst} 5\,C_2F_5I$

2) $C_2F_5I + nC_2F_4 \rightarrow C_2F_5(C_2F_4)_nI$

3) $C_2F_5(C_2F_4)_nI + C_2H_4 \rightarrow C_2F_5(C_2F_4)_nC_2H_4I$

Fig. 3. Example of the telomerization process.

oroalkylethyliodides are synthesized via the reactions shown in Fig. 3. In the second stage, the iodide is replaced with a functional group, depending on the application. The primary building block is 1*H*,1*H*,2*H*,2*H*-perfluorodecanol (8:2 FTOH; Fig. 4), which is produced with monomethylformamide (AtoFina 2001). In contrast with the ECF process, telomerization produces only linear products, which can contain small amounts of shorter carbon chain compounds (Kissa 2001).

III. Environmental Fate and Occurrence

A. General Use

PFAS are used for a wide variety of applications. The quantitatively most important ones are as follows (USEPA 2002a; NCEHS 2001; DuPont 2002):

1. Carpet protection
2. Textile protection
3. Leather protection
4. Paper and board protection
5. Fire-fighting foams
6. Specialty surfactants, e.g., cosmetics, electronics, etching, medical use, plastics
7. Polymerization aid

Other niche markets include antifogging, cement additives, herbicides, and oil wells (Kissa 2001).

In the former three applications listed above, the PFAS are used as a copolymeric coating to provide water, grease, and soil repellency to the treated fiber/material (Kissa 2001). The low surface energy of the perfluoroalkylated chains, and the water and grease repellency of these compounds, protects the fibers by

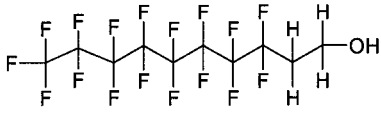


Fig. 4. Chemical structure of 1*H*,1*H*,2*H*,2*H*-perfluorodecanol (8:2 FTOH).

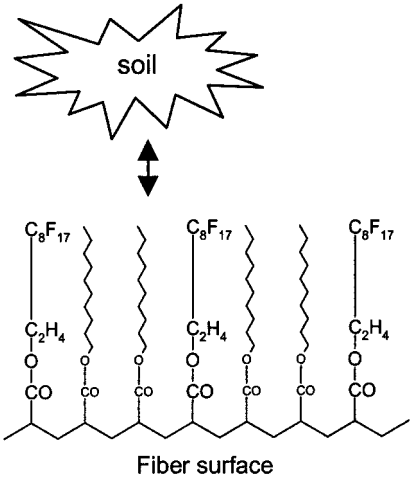


Fig. 5. Creation of soil-repellent layer of perfluoroalkylated substance- (PFAS-) treated carpet.

an impermeable external layer, in a manner shown in Fig. 5 (Tomasino 1992). For paper protection, the same mechanism of protection is available. Most of the commercial products that are used in this type of application contain perfluoroalkyl phosphates as the active ingredient (Fig. 6; Kissa 2001). The latter three categories of application listed here use PFAS in the monomeric form (Moody and Field 2000; NCEHS 2001; Kissa 2001). An example of these substances is presented in Fig. 7. Pabon and Corpart (1999) and Moody and Field (2000) have reviewed the use of PFAS in fire-fighting foams.

B. Use and Emissions in the Netherlands

The use of PFAS leads to their emission to the environment. An estimation was made of the emissions resulting from the use of PFAS in the Netherlands (Table 2). To that end, for the seven categories of application mentioned an effort was made to retrieve use data for the Dutch market. These data are presented in Table 2. The numbers represent the amount of PFAS in the used products. For a comparison, the use data for these chemicals in the United Kingdom, collected in a recently conducted inventory (NCEHS 2001), are also shown.

A recent evaluation of possible emissions from PFAS-treated products during

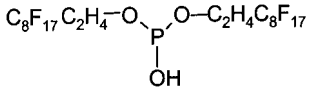


Fig. 6. General structure of perfluoroalkyl phosphates.

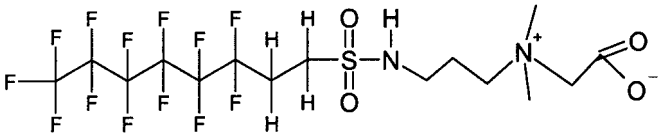


Fig. 7. Example of monomeric PFAS: perfluoroalkylbetaine used in fire-fighting foams.

the entire life cycle estimated that a very large amount of the applied copolymeric and phosphatic PFAS will wear from the fibers, leading to substantial emissions of PFAS to the environment (3M 2000c). In addition, the use of PFAS-treated paper might lead to emissions to the environment when the waste is landfilled. Currently, there are no study results available that can either confirm or disaffirm this assumption. In the Netherlands, approximately 39% of the waste is landfilled (Milieuloket 2001). In a worst case estimation, this would correspond to an emission from paper and paperboard use of 23–41 tonnes of PFAS annually. Use of PFAS in fire-fighting foams can lead to direct emissions to the environment, as has been discussed by Moody and Field (1999, 2000) and Moody et al. (2001, 2002). The emissions from the use of speciality surfactants could not be assessed because use data for these applications in the Netherlands are lacking.

The use of PFAS as a polymerization aid may also give rise to their emission to the environment. The Association of Plastic Manufacturers Europe (APME) estimated that in global fluoropolymer production 61% of the polymerization aid is emitted to water, air, and land and that 16% is still present in the produced polymer (APME 2002). Hansen et al. (2002) have demonstrated that the effluent of a fluorochemical manufacturing plant can be a source of PFAS in the environ-

Table 2. Estimated use and emissions of perfluoroalkylated substances (PFAS) in The Netherlands (NL) and the UK.

Type of industry	Use NL (tonnes/yr)	Use UK (tonnes/yr)	Form	Emissions NL (tonnes/yr)[a]
Carpet	15	195[b]	Polymers	10 (mostly wear)
Paper and board	60–105 (not in NL)	60	Phosphates	23–41 (landfilled waste)
Textile	N.A.	—[b]	Polymers	N.A. (100%, mostly wear)
Leather	10–20	N.A.	Polymers	10–20 (mostly wear)
Fire-fighting foams	1–4	65	Monomers	1–4 (use)
Specialty surfactants	N.A.	70	Monomers	N.A.
Polymerization aid	>1	N.A.	Monomers	>0.77

Source: Hekster et al. (2002).
N.A.: not available.
[a]These figures represent a worst case estimation.
[b]Carpet and textile together.

ment. Consequently, both the production and the use of fluoropolymers can lead to the emission of PFAS to the environment. Fluoropolymers are produced in the Netherlands, and these may lead to the emission of the polymerization aid (PFOA) used in the production process. No PFAS production plants are present in the Netherlands, but the emissions from a plant in Antwerp, Belgium, may lead to emissions to the adjacent Dutch Scheldt estuary and coastal environment. Results of an environmental monitoring study have indeed revealed relatively high concentration of PFOS in the Scheldt estuary, downstream from Antwerp, and adjacent North Sea (Van de Vijver et al. 2002; Van de Vijver et al., in manuscript). Additional sources of fluvial and aerial influx of these compounds may stem from the use of PFAS in textile and paper factories close to the Belgian and German borders.

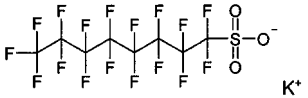
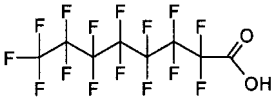
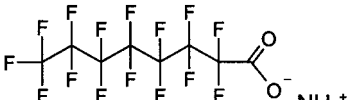
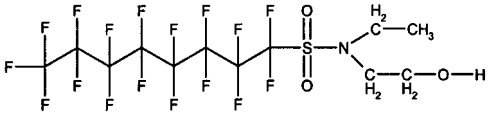
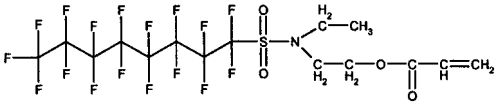
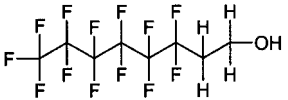
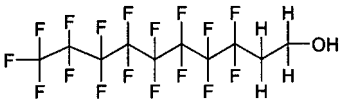
Although the numbers presented in this section are rough estimations, and some data are obviously missing for a complete evaluation, it can be concluded (see Table 2) that in the Netherlands annually several tens of tonnes of PFAS may be emitted to the environment.

C. Environmental Fate

There is a paucity of data on the psychochemical properties of perfluoroalkylated substances. Although the first reported measurements of such data date back to 1976 (3M 1976), most research efforts to obtain reliable data necessary for the evaluation of the environmental fate of PFAS have only started relatively recently, and many essential data are still lacking. It has become clear, however, that PFAS behave differently from nonpolar and slightly polar organic micropollutants. Because the perfluoroalkylated chain is oleophobic and hydrophobic (Key et al. 1997), PFAS neither accumulate in fatty substances nor sorb to organic matter solely due to hydrophobic interactions. Fluorosurfactants are intrinsically polar chemicals. For example, PFOS is present in the environment as the dissociated salt (3M 2001b). Therefore, electrostatic interactions may play an important role in their distribution. Both biotic membranes and sediment surfaces have various polar parts with which such interactions are plausible. For these reasons, the prediction of environmental behavior through quantitative structure–activity relationships (QSAR) based on octanol–water partitioning is not applicable to PFAS.

From the data that are available, it appears that large differences exist between the solubility, vapor pressure, and Henry's law constant data for the various PFAS (Table 3). Consequently, large differences in environmental behavior may be expected. For PFOS and PFOA, the combination of their high aqueous solubility and low vapor pressure, resulting in low Henry's law constants, makes it unlikely that they will be transported through air over large distances (Renner 2001; Martin et al. 2002). On the other hand, *N*-EtFOSE, 6:2 FTOH, and 8:2 FTOH have low solubilities. Combined with a moderately low vapor pressure, the latter chemicals have a tendency to escape from the water phase to air and to be transported over a long range by air. The latter, of course, also depends

Table 3. Environmentally relevant properties of selected PFAS.

Substance	Aqueous solubility (g/L)	P_{vapor} (Pa)	H[a] (atm m^3 mol^{-1})	Chemical structure
PFOS (K^+)	5.19 E-1	3.31 E-4	3.4 E-9	
PFOA (H^+)	9.5	7.0 E1	4.6 E-6	
PFOA (NH_4^+)	>5.00 E2	<1.3 E-3/9.2 E-3	<1.1 E-11/7.8 E-11	
N-EtFOSE	1.51 E-4	5.04 E-1	1.9 E-2	
N-EtFOSEA	8.9 E-4	N.A.	—	
6:2 FTOH	1.2–1.7 E-2	N.A.	~1 E-2	
8:2 FTOH	1.40 E-4	2.93	9.6 E-2	

Source: Hekster et al. (2002). PFOA: perfluorooctanoic acid; PFOS: perfluorooctyl sulfonate; *N*-EtFOSE: *N*-ethylperfluorooctane-sulfonamidoethanol; *N*-EtFOSEA: *N*-ethylperfluorooctanesulfonamidoethyl acrylate; 6:2 FTOH: 1*H*,1*H*,2*H*,2*H*-perfluorooctanol; 8:2 FTOH: 1*H*,1*H*,2*H*,2*H*-perfluorodecanol; N.A.: not available. [a]H: Henry's law constant (calculated values)

on other factors such as photochemical stability and scavenging rates. However, no experimental data are presently available for these properties.

The sorption of PFOS to sediment and sludge has been shown to be strong, whereas no ready desorption was observed (3M 2001c). For PFOA and other PFAS, no reliable conclusion could be drawn yet with respect to sorption to soil and sediment. For telomers, no environmentally relevant test data are available, but adsorption to laboratory equipment was very high and desorption very difficult, suggesting comparable strong sorption to environmental media (TRP 2002).

The transformation of the perfluoroalkylated part of PFAS is expected to proceed very slowly because the fluorine–carbon bond is the strongest single bond with carbon (Smart 1994). The available data show that only the nonfluorinated part of the ECF products can be degraded by microorganisms into PFOS, via an aerobic route, or, to a lesser extent, into PFOA, via an anaerobic route. The latter two chemicals do not appear to be degraded any further (3M 1976, 1978a, 2000d–g, 2001d). None of the tested fluorosurfactants (PFOS, PFOA, *N*-EtFOSE, 8:2 FTOH) appeared to be vulnerable to direct photolytic attack (3M 1979a,b, 1981a, 2001e; TRP 2002). However, indirect photolysis in air through reactions with OH^- radicals may play a role in the decomposition of fluorinated chemicals (Vésine et al. 2000). The fluoroalkyl chain of all ECF products tested experimentally was not affected by hydrolysis.

Only one literature source dealing with the degradation of telomer products in the environment is available. This study showed that, during the degradation of 1*H*,1*H*,2*H*,2*H*-perfluorooctane sulfonate under sulfur-limiting conditions by *Pseudomonas* sp. strain D2, volatile degradation products were formed containing carbon, oxygen, hydrogen, and fluorine. Furthermore, the detection of fluoride indicated defluorination (Key et al. 1998). Current research is dedicated to the biodegradation routes of telomer products (Renner 2001; TRP 2002).

As discussed in the emissions section, copolymeric PFAS may be emitted to the environment. The environmental fate of these substances has not been studied. In general, polymeric substances cannot cross membranes, and therefore will elicit very minor toxic effects, if any. If, however, monomeric PFAS would be formed (e.g., by wear) from (co-)polymeric substances, this degradation could have huge environmental implications. In interviews with manufacturers, it was suggested that fluorinated organic polymers are very stable (3M 2002; Bayer 2002). 3M states that they "[. . .] have data demonstrating the stability of high molecular weight fluorochemical polymers and phosphate esters to various mechanisms of degradation" (3M 2000h). One study on the hydrolytic stability of fluoropolymers is available. Although the data of this study have to be treated with caution, because of its limited reliability, they showed that fluorinated organic polymers are rather stable to hydrolysis, resulting in half-lives ranging from 1 to 5 yr for acrylates and esters to 500 yr for fluorinated urethanes (3M 2000i).

From a chemical point of view, hydrolysis of the ester bond in (co-)polyacrylates and (co-)polymethacrylates seems possible, leading to the formation of

perfluorinated alcohols, which may react further to form perfluorinated carboxylic acids (this mechanism is explained in Fig. 8). Also, the stability of the phosphoryl bond in the fluoroalkyl phosphates is not well known. The cleavage of this bond could lead to the formation of fluoroalcohols.

When discharged to water, PFOS partially sorb to soil and sediment. In addition, bioconcentration of PFOS is likely to occur (Martin 2002; Martin et al. 2003a). Therefore, water, sediment, and biota are believed to be the most important compartments for the environmental fate of PFOS. PFOA does not evaporate from the water phase, and its sorption potential is not clear. The bioconcentration factor for PFOA is 3.2–25 (see Section IV.A). Therefore, water is believed to be the target compartment for PFOA.

D. Environmental Occurrence

In the previous section, it was shown that PFOS and PFOA are persistent degradation products of PFAS produced by the ECF process. The degradation pathways in the environment of PFAS produced by telomerization are currently unknown. In an experimental study with rats it was shown that a telomer alcohol was transformed into PFOA (Hagen et al. 1981; cf. Section IV.B). The significance of this finding for the environmental occurrence of PFOA remains to be elucidated. It is expected that PFAS are present in the environment primarily in the form of their degradation products. Therefore, the discussion of the environmental occurrence and toxicity of PFAS here is focused on PFOS and PFOA.

In a 3M study (3M 2001c), PFAS were determined in several media from six urban areas in the United States. PFOS was detected most often, followed by PFOA and FOSA, all at relatively low concentrations, ranging from below limit of quantification (LOQ) to 2980 μg/L. The highest concentrations were found in sewage sludge. Other important media included sewage treatment plant effluent and landfill leachate. The highest concentrations were observed in cities where PFAS were manufactured or industrially used, but PFAS were also pres-

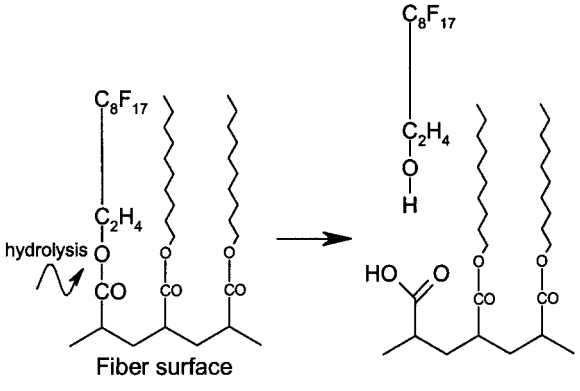


Fig. 8. Formation of monomeric PFAS from (co-) polymeric substances.

ent in various media sampled in control cities. Hansen and coworkers (2002) also showed that elevated PFAS concentrations were found in the Tennessee River (up to 0.6 μg/L of PFOS and 0.15 μg/L for PFOA) from emissions from a fluorochemical manufacturing plant.

PFAS can occur in groundwater as a result of the use of fire-fighting foams. PFAS concentrations in groundwater were found to vary with time and distance from the use of the fire-fighting agent (Levine et al. 1997; Moody and Field 1999). Quantitatively, PFOA and PFOS were the most important fluorochemicals detected in groundwater. Six PFAS, including three ECF products and three telomers, have been detected in the air of a highly urbanized site in Toronto, Canada. The concentrations ranged from 14 pg/m^3 (*N*-EtFOSA) to 205 pg/m^3 (*N*-EtFOSE). Air concentrations monitored in the same study at a rural site in Canada were between 1.7- and 2.9 fold less; five of the six PFAS determined could be quantified at this location (Martin et al. 2002).

Concentrations of PFOS were detected in marine and estuarine biota from the Western Scheldt and the adjacent Belgian coastal zone of the North Sea. Average concentrations ranged from 36 ng/g to 1.7 μg/g (Van de Vijver et al. 2002; Hoff et al. 2003). These results may not be representative for coastal and marine environments, because a factory producing fluorochemicals located upstream from the Scheldt estuary may cause elevated downstream PFAS concentrations.

Recently, Kannan and coworkers published a series of papers on the occurrence of PFOS and related chemicals in wildlife. More than 900 samples were analyzed, including blood, liver, and muscle samples of European and North American marine mammals, birds, and fish, and North American mink, otter, turtles, and frogs. Furthermore, various marine mammals and birds from remote locations were sampled (Giesy and Kannan 2001; Kannan et al. 2001a,b, 2002a–d). All samples collected contained PFOS above the limits of detection. Large differences between individual species were observed. No clear age- or sex-related differences could be observed (Kannan et al. 2001a, 2002b–d). For all species, the PFAS concentrations observed were invariably higher in animals from more urbanized or industrialized areas.

In Fig. 9, PFOS concentrations of comparable species taken from the studies by Kannan et al. have been grouped and averaged according to the origin of the samples. It is evident that the concentrations in remote locations are still very much lower than concentrations in Europe or the U.S. The concentrations of PFOS found in livers of animals from the U.S. were higher for dolphin, cormorant, and bald eagle than in similar species from Europe, whereas the contrary was observed for seals: European seals contained higher levels of PFOS in liver than seals from the U.S.

Human exposure to organic fluorine has been observed as early as 1968. Taves (1968) concluded that "[. . .] if in fact there is a non-exchangeable fluoride in serum, it did not break down or diffuse under these conditions, implying a large stable molecule. These findings are consistent with the presence of a fluorocarbon molecule." With the development of analytical methods in recent

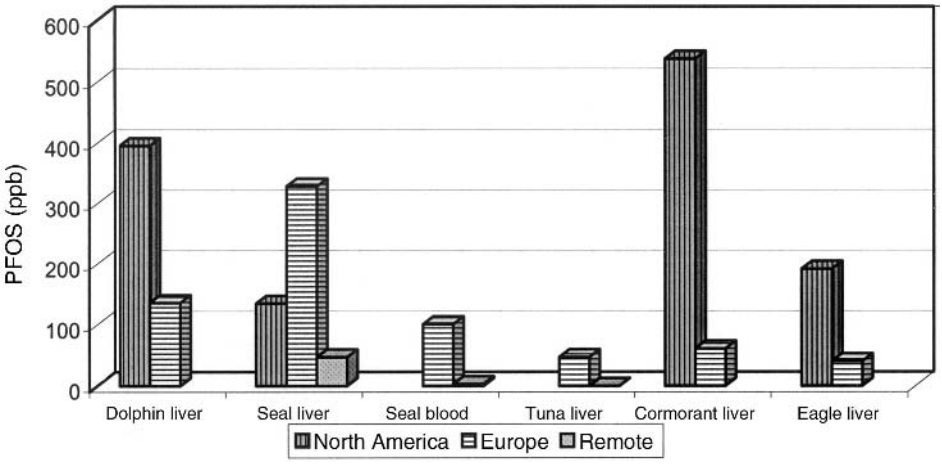


Fig. 9. Comparison of PFOS concentrations between samples from North America, Europe, and remote locations. Mean concentrations are given in ng/g wet weight for liver and ng/mL for blood. (From Giesy and Kannan 2001; Kannan et al. 2001a,b.)

years, the identification of organic fluorine compounds has improved. Although there has been some debate on the origin of organic fluorine in humans (Belisle 1981), it is now generally accepted that there is an anthropogenic origin.

Since 1993, several studies have been performed on the occurrence of PFAS in humans. Both Olsen and coworkers and Gilliland and Mandel published two studies on levels of PFOS and PFOA in production workers occupationally exposed (Gilliland and Mandel 1993, 1996; Olsen et al. 1999, 2000). They reported that PFOS and PFOA accumulated in human serum and liver. Concentrations of PFOS and PFOA in serum from occupationally exposed workers were in the 1–2 mg/L range. To compare these data with the general population, blood from people nonoccupationally exposed was also analyzed. Pooled serum samples from blood dating as far back as 1957 showed concentrations of several tens of μg/L (OECD 2002). Samples from 1998–2000 showed average serum levels of 17–53 μg/L for PFOS and 3–17 μg/L for PFOA. No differences could be observed between children (37.5 μg/L) and elderly people (31 μg/L).

IV. Toxicity

A. Bioaccumulation

The degree of bioaccumulation is an important factor in the overall environmental risk of a compound. As was discussed in Section III.C, this cannot be modeled by using the octanol–water partition coefficient.

The bioaccumulation factor for PFOS for the common shiner (a fish) was reported to vary between 6,300 and 125,000 (Moody et al. 2002). The clearance was reported to take more than 130 d (3M 2000l). Martin et al. (2003a,b) deter-

mined experimental bioaccumulation, biomagnification, and bioconcentration data for seven ECF-type perfluoroalkylated substances (carboxylates and sulfonates) in blood, liver, and carcass of rainbow trout. These authors argued that in trout the bioaccumulation is solely due to bioconcentration, because biomagnification factors (BMF) for all tested PFAS were less than 1. The observed bioconcentration factor (BCF) of PFOS for rainbow trout after 12 d of exposure was reported to be between 690 (carcass) and 3,100 (blood). Corresponding steady-state BCFs were slightly (i.e., a factor of 1–2) higher. The highest BCFs observed in this study amounted to 20,000 for the accumulation of the tetradecanoic homologue of PFOA in blood.

Bioconcentration factors of PFOA in rainbow trout varied from 3.2 in carcass to 25 in blood (Martin et al. 2003a). Clearance of PFOA from fathead minnows took more than 15 d (3M 1995). For 8:2 FTOH, a water concentration-dependent bioconcentration factor was determined, varying from 87–310 (at 1 μg/L exposure concentration) to 200–1100 (at 10 μg/L) (TRP 2002). No data for the removal rate were available.

The extent of bioconcentration of PFAS appears to be highly structure dependent. Martin et al. (2003a) showed that carboxylates with less than 7 and sulfonates with less than 6 perfluoroalkyl carbons did not accumulate in rainbow trout. For longer PFAS, bioconcentration factors increased with increasing length of the perfluoroalkyl chain, ranging from 4 to 23,000. Sulfonates accumulated to a greater extent than corresponding carboxylates.

B. Biotransformation

Degradation and transformation of PFAS was discussed briefly in Section III.C. Transformation of the perfluoroalkyl chain of PFAS occurs very slowly, if at all, because of the strong C–F bond. Thus, PFOS and PFOA appear to be stable end products of (bio)degradation of PFAS (Key et al. 1998). PFOS and PFOA are not metabolized in biota (OECD 2002; USEPA 2002b), which is probably due to the stability of the C–F bond. Similarly, biotransformation of telomers may lead to stable PFAS intermediates. Indeed, Hagen et al. (1981) reported the qualitative biotransformation of 8:2 FTOH into PFOA in rats. Microbial degradation of the telomer tetrahydrogen-substituted PFOS resulted in partial defluorination (Key et al. 1998).

C. Mechanisms of Toxicity

The mechanisms of toxicity of individual perfluoroalkylated substances are not well understood. The perfluorocarboxylates, including PFOA, are peroxisome proliferators (Intrasuksri et al. 1998). The same mechanism of toxicity has been suggested for several other PFAS (Giesy and Kannan 2002).

D. Ecotoxicity

The toxicity to aquatic organisms of several PFAS has been investigated in several studies. Most of these have been performed with ECF products and PFOS and PFOA in particular. However, many of the studies have followed

protocols that deviate from the current standard methodologies as defined by the OECD (1992) and the European Chemicals Bureau (ECB 1996). The most important deviations include limited purity of the test chemical and the use of nominal concentrations instead of measured concentrations. The lowest observed $E(L)C_{50}$ and NOEC values for toxicity to aquatic organisms obtained in studies considered reliable are presented in Table 4. These studies comply with the protocols of the ECB or OECD.

For the classification of toxicity data, the categorization as developed by Van Rijn and coworkers (1995) is followed. The data in Table 4 show that PFOS is moderately acutely toxic and slightly chronically toxic to aquatic organisms, PFOA is both acutely and chronically practically nontoxic, and *N*-EtFOSA is slightly acutely toxic to daphnids. For 8:2 FTOH, no conclusion can be drawn, because the published NOECs all correspond to concentrations slightly above the reported aqueous solubility (cf. Table 3) for this compound. Further research is necessary to confirm the reliability of these data. Several studies on the chronic toxicity of 8:2 FTOH are underway (TRP 2002).

The toxicity to marine organisms has also been tested for PFOS. PFOS appeared to be moderately acutely toxic and slightly chronically toxic to invertebrates (3M 2000l,m).

E. Ecological Risk Assessment

Based on the toxicity data presented here, an indicative maximum permissible concentration (iMPC) can be derived (Traas 2001), following the methodology of the European Chemicals Bureau (ECB 1996) or the modified EPA method, using safety factors (OECD 1992). For PFOS an iMPC of 5 μg/L has thus been calculated in the present study, using a safety factor of 50 and the lowest chronic EC_{50} value (see Table 4), being the 35-d NOEC for marine mysid shrimp. The highest concentration that was observed in the quoted multicity environmental monitoring study (3M 2001c) amounted to 5.0 μg/L for PFOS in sewage treatment plant effluent from a city with a fluorochemical plant. Surface water concentrations were generally much lower. The highest concentration of PFOS observed in freshwater from a control city was 2.2 μg/L (3M 2001c). The highest concentration of PFOS in water reported after an accidental fire-fighting foam spill was 2210 μg/L (Moody et al. 2002). These values indicate that the iMPC for PFOS may be approached in urban areas.

For PFOA, an iMPC of 300 μg/L was derived, using a safety factor of 1000 and the lowest acute EC_{50} value (see Table 4; 300 mg/L for *P. promelas*). The highest freshwater concentrations observed in the multicity environmental monitoring study corresponded to 2.3 μg/L for PFOA in sewage treatment plant effluent from a city with a fluorochemical plant. The highest reported concentration of PFDA in water from control cities was 0.75 μg/L (3M 2001c). The highest concentration reported for PFOA in groundwater at a fire-fighting training site was 6570 μg/L (Moody and Field 1999). These values indicate that the iMPC for PFOA may only be exceeded after spills.

Table 4. Summary of reliable, lowest observed E(L)C_{50} and NOEC values for PFAS.

Substance	Acute/chronic	Trophic level	Species	Results (mg/L)	Reference
Freshwater toxicity					
PFOS	Acute	Algae	*Selenastrum capricornutum*	72 hr EC_{50} = 120	3M (2000j)
		Invertebrates	*Daphnia magna*	48 hr EC_{50} = 58	Panarctic Oil (1986)
		Fish	*Oncorhynchus mykiss*	96 hr EC_{50} = 7.8	Panarctic Oil (1986)
	Chronic	Invertebrates	*Daphnia magna*	28 d NOEC = 7	3M (1984)
		Fish	*Pimephales promelas*	42 d NOEC = 0.30	3M (2000k)
PFOA	Acute	Bacteria	*Photobacterium phosphoreum*	30 min EC_{50} = 722	3M (1987a)
		Algae	*Selenastrum capricornutum*	96 hr EC_{50} > 1000	3M (1996)
		Fish	*Pimephales promelas*	96 hr LC_{50} = 300	3M (1987b)
	Chronic	Algae	*Selenastrum capricornutum*	14 d EC_{50} = 43	3M (1981b)
		Fish	*Pimephales promelas*	30 d NOEC > 100	3M (1978b)
8:2 FTOH	Acute	Algae	*Scenedesmus subspicatus*	72 hr NOEC = 0.20	DuPont (2002)
		Invertebrates	*Daphnia magna*	48 hr NOEC = 0.16	DuPont (2002)
		Fish	*Danio rerio*	96 hr NOEC = 0.18	DuPont (2002)
N-EtFOSA	Acute	Invertebrates	*Daphnia magna*	48 hr EL_{50} = 14.5	3M (1998a)
		Fish	*Pimephales promelas*	96 hr LL_{50} = 206	3M (1998b)
Marine toxicity					
PFOS	Acute	Algae	*Skeletonema costatum*	96 hr EC_{50} > 3.2	3M (2001g)
		Invertebrates	Mysid shrimp	96 hr EC_{50} = 3.6	3M (2000l)
		Fish	Sheepshead minnow	96 hr LC_{50} > 15	OECD (2002)
	Chronic	Invertebrates	Mysid shrimp	35 d NOEC = 0.25	3M (2000m)

As stated in the preceding section, carboxylates and sulfonates with short perfluoroalkyl carbon chains do not appear to bioconcentrate or biomagnify in rainbow trout. For longer homologues, bioconcentration factors increase with increasing perfluoroalkyl chain length. Therefore it is likely that the environmental risk of short chain carboxylates (<7) and sulfonates (<6) is less than that of PFOA and PFOS, whereas longer homologues could imply higher risks. Data on both the ecotoxicity and the environmental concentrations of these homologues are currently lacking, thus hampering any firm conclusion to be drawn at this moment.

F. Human Toxicity

The human toxicity of PFOA and to a lesser extent of PFOS has been, and still is, the subject of many studies. For the telomers few data are available but more studies are underway (TRP 2002).

Both PFOS and PFOA have long half-lives (8.67 yr and 1–3.5 yr, respectively) in the human body. The excretion from the body is slow and occurs via urine and feces. Both chemicals are distributed to liver, plasma, and kidney. PFOA binds covalently to macromolecules. Perfluorocarboxylic acids with a longer perfluoroalkyl chain are less easily excreted from the body (Kudo et al. 2001). PFOS and PFOA exhibit low acute toxicity to rodents but are eye irritating (OECD 2002; USEPA 2002b).

In chronic feeding tests with rodents and primates, the primary target for PFOS and PFOA was the liver. PFOA was found to be weakly carcinogenic. Mutagenicity testing of PFOS did not show any mutagenic effects. PFOA induced chromosomal aberrations and polyploidy in Chinese hamster ovary (CHO) cells, but did not show mutagenic effects in all other mutagenicity tests that were conducted, including an in vivo micronucleus test. All tested telomers (6:2, 8:2, 10:2, and 12:2 FTOH) reacted negatively in various mutagenicity tests (DuPont 2002; OECD 2002; USEPA 2002b). In a developmental effect study with PFOS the no observed adverse effect level and the lowest observed adverse effect level for the second generation of rodents were determined to be 0.1 mg/kg/d and 0.4 mg/kg/d, respectively (TRP 2002).

V. Conclusions and Recommendations

Perfluoroalkylated substances are used in large amounts for various applications. Emissions to the environment are inevitable from some of these applications. In the Netherlands, presumably several tens of tonnes of PFAS are emitted annually, which correspond to a large amount of the annual use. There are no reasons to assume that the use/emission ratio for each type of application would be much different for other European or North American countries.

After emission, the ECF products are probably degraded to PFOS and PFOA. Only one study on the degradation of telomers is available, also showing a possible biotransformation to PFOA. Therefore, the focus for evaluating the

environmental impact of PFAS is placed on PFOS and PFOA. The perfluoroalkyl chain of these chemicals is not vulnerable to further photolytic or hydrolytic attack.

Because many PFAS are applied as (co-)polymers, the possible degradation products of these polymers are of interest. Although no reliable tests have been performed, it seems possible that the ester bond in acrylates can be broken, leading to the emission of monomeric PFAS to the environment. Future investigations should address this important aspect of fluorinated polymers. PFOS and PFOA are unlikely to be transported by air over long distance for reason of their low Henry's law constant. Several other PFAS have the tendency to leave the water phase and partition to air. Thus, they may be prone to being transported over long distances. Some of these chemicals can be degraded to form PFOS or PFOA, which can be an important mechanism in the global distribution of PFOS and PFOA. PFOS, and to a lesser extent PFOA, have been detected in various vertebrate species from around the globe, primarily in liver and blood. Concentrations were higher in animals from more industrialized or urbanized areas. It is not likely that PFOA itself is accumulating in biota, because of its reported low experimental BCF and BMF. The origin of PFOA in biota may be transformation of both ECF products and telomers within the body after their accumulation. Future research should focus on elucidating more clearly the pathways that lead to PFOS and PFOA accumulation in biota.

Experimental BCFs range from relatively high for PFOS (690–3100) to low for PFOA (3.2–25), whereas for 8:2 FTOH a concentration-dependent BCF was found, 87–310 at an exposure concentration of 1 μg/L and 200–1100 at 10 μg/L. Removal rates for PFOS and PFOA from biota are low. Short alkyl chain perfluorinated acids (<7) and sulfonates (<6) do not accumulate. Above a perfluoroalkyl chain length of 7 carbons, the bioconcentration factor increases with the chain length. None of the tested PFAS showed biomagnification factors higher than 1.

The freshwater and marine acute toxicities of all PFAS tested can be qualified as moderately toxic or less. The freshwater and marine chronic toxicities reported can be qualified as slightly toxic or less. The available toxicity data have been used to derive an indicative maximum permissible concentration (iMPC) in water. For PFOS, this value is 5 μg/L. Concentrations close (within a margin of a factor of 3) to this level have been observed in effluents and surface water from urbanized areas in the United States. Locally, due to spills from fire fighting, concentrations may significantly exceed this iMPC value. For PFOA an iMPC of 300 μg/L was derived. Environmental monitoring studies indicate that this value can only be exceeded locally and temporarily due to spills.

Humans occupationally exposed to PFAS have much higher PFOS and PFOA concentrations than the general public. Serum levels for fluorochemical plant workers are in the 1–2 mg/L range. The general public has serum levels of 17–53 μg/L for PFOS and 3–17 μg/L for PFOA. In humans, PFOS and PFOA have long half-lives and are distributed to liver, plasma, and kidney.

Acute toxicity of PFOA and PFOS to rodents is low. Mutagenicity testing with PFOS did not demonstrate any mutagenic effects. PFOA was found to be weakly carcinogenic and reacted negatively in most of the mutagenicity tests. All tested telomers reacted negatively in various mutagenicity tests.

Summary

The production, use, environmental fate, occurrence, and toxicity of perfluoroalkylated substances have been reviewed. Although only a limited number of essential physicochemical data are available, thus hampering a complete assessment of the environmental fate of PFAS, it has become clear that PFAS behave differently from other nonpolar organic micropollutants. PFAS are present in environmental media in urbanized areas both with and without fluorochemicals production sites. The presence of PFOS at levels above the limit of detection has been demonstrated in almost all organisms sampled in a global survey as well as in both nonexposed and exposed human populations. The acute and chronic ecotoxicity of PFOS, PFOA, and 8:2 FTOH to aquatic organisms is moderate to low. Acute toxicity to rodents is also low. PFOS concentrations in effluents have been reported that approach indicative target values derived from available aquatic toxicity data. PFOA has been found to be weakly carcinogenic. This review shows the importance of the perfluoroalkylated substances for the environment and the necessity to fill the current gaps in knowledge of their environmental fate and effects.

Acknowledgments

The authors thank the National Institute for Coastal and Marine Management (RIKZ) for funding and A.M.C.M. Pijnenburg for her useful advice. The Telomer Research Program, the United States Environmental Protection Agency, the 3M company, and many individual company employees and scientists are gratefully acknowledged for sharing their data with us.

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Manuscript received December 7; accepted December 18, 2002.

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Reviews of Environmental Contamination and Toxicology

Edited by
George W. Ware

Published by
Springer-Verlag New York · Berlin · Heidelberg · Hong Kong
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Abbreviations

A	acre	min	minute(s)
bp	boiling point	*M*	molar
cal	calorie	mon	month(s)
cm	centimeter(s)	ng	nanogram(s)
d	day(s)	nm	nanometer(s)(millimicron)
ft	foot (feet)	*N*	normal
gal	gallon(s)	no.	number(s)
g	gram(s)	od	outside diameter
ha	hectare(s)	oz	ounce(s)
hr	hour(s)	ppb	parts per billion (µg/kg)
in.	inch(es)	ppm	parts per million (mg/kg)
id	inside diameter	ppt	parts per trillion (ng/kg)
kg	kilogram(s)	pg	picogram(s)
L	liter(s)	lb	pound(s)
mp	melting point	psi	pounds per square inch
m	meter(s)	rpm	revolutions per minute
m^3	cubic meter(s)	sec	second(s)
µg	microgram(s)	sp gr	specific gravity

µL	microliter(s)	sq	square (as in "square m")
µm	micrometer(s)	vs	versus
mg	milligram(s)	wk	week(s)
mL	milliliter(s)	wt	weight
mm	millimeter(s)	yr	year(s)
m*M*	millimolar		

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