



C



H

EVALUATION OF CURRENT EMISSIONS OF CHLORINATED



L



O

MICROCONTAMINANTS FROM THE DUTCH CHLORINE CHAIN



R



I

B. van Hattum
H. Senhorst
A. Tukker

M. Lamoree
T. Sanderson
A. de Koning



N



E

Evaluation of current emissions of chlorinated microcontaminants from the Dutch chlorine chain

B. van Hattum¹, H. Senhorst², A. Tukker³, M. Lamoree¹, T. Sanderson⁴
and A. de Koning⁵

¹ IVM, Vrije Universiteit, Amsterdam

² RIZA, Rijkswaterstaat, Lelystad

³ TNO-STB, Delft

⁴ IRAS, Universiteit Utrecht

⁵ CML, Universiteit Leiden

Final Technical Report of the Chlorine Chain Follow-up Research Programme on Chlorinated Organic Microcontaminants (OVOC)

E-04/08

November 2004

Commissioned by the Ministry of Housing, Spatial Planning and the Environment (VROM, DGM; contract nr. 99230300), the Ministry of Transport, Public Works and Water Management (V&W, Directorate General for Water Affairs), the Association of the Dutch Chemical Industry (VNCI) and the Netherlands Society for Nature and Environment (SNM).

This report was approved by the OVOC steering committee (28 September 2004).

IVM

Institute for Environmental Studies

Vrije Universiteit

De Boelelaan 1087

1081 HV Amsterdam

The Netherlands

Tel. ++31-20-4449 555

Fax. ++31-20-4449 553

E-mail: info@ivm.falw.vu.nl

Copyright © 2004, Institute for Environmental Studies

All rights reserved. No part of this publication may be reproduced, stored in a retrieval system or transmitted in any form or by any means, electronic, mechanical, photocopying, recording or otherwise without the prior written permission of the copyright holder.

Contents

Preface	iii
Abstract	v
Executive Summary	vii
Part 1 Introduction and Background	1
1. General introduction	3
1.1 Context and framework	3
1.2 Scope and objectives	5
2. Chlorinated microcontaminants: priorities and other background studies	7
2.1 Priority setting and selection of sources	7
2.2 Studies facilitating interpretation of experimental results	9
Part 2 Experimental Studies	15
3. Introduction	17
3.1 Objectives of the OVOC project	17
3.2 Selection of toxicity-screening tests	19
3.3 Criteria and screening methods for PBT characteristics	20
3.4 Dioxin-like compounds and biological/chemical assessment methods	21
3.5 Identification of substances	25
4. Description of experimental methods	27
4.1 Framework	27
4.2 Sampling programme	28
4.3 Biological methods	29
4.4 Status of <i>in vitro</i> methods applied in study	31
4.5 Fractionation of extracts	32
4.6 GC-MS identification studies	32
4.7 Persistency-testing of aquatic effluents	33
5. Results and discussion	37
5.1 Chlorinated substances identified in emissions and products	37
5.2 Toxicity screening of emissions and products	40
5.3 Linking identification and effects	44
5.4 Loads of substances and toxic-equivalents	47
5.5 PBT character of identified chlorinated substances	51
5.6 Discussion of <i>in vivo</i> results	57
5.7 Discussion of <i>in vitro</i> results	58
Part 3 Synthesis and Conclusions	63

6. Synthesis	65
6.1 Addressing the goals of the OVOC project	65
6.2 Methodological limitations relevant for conclusion	70
6.3 Findings in light of recent emission reduction programmes	72
6.4 Remaining uncertainties concerning by-product management	73
7. Conclusions	75
Part 4 References and Appendixes	79
8. References	81
9. Appendixes	87
9.1 Priority-setting procedure for sampling programme	88
9.2 Studies facilitating the interpretation of results	99
9.3 Publications and reports from OVOC project	104
9.4 Summary information on DR-CALUX and Carp-Hep assays	106
9.5 PBT definitions in regulatory frameworks	108
9.6 Analysis of GC-MS data and identification with AMDIS-NIST	110
9.7 In-plant TIE study	114
9.8 Biodegradation study	116
9.9 Extensive analysis of DR-CALUX response in MCD samples	118
9.10 Glossary (abbreviations)	120
9.11 BOVOC steering committee participants	124
9.12 List of Tables and Figures	125

Preface

OVOC programme

The present report contains an extensive summary and synthesis of the final results of the Dutch research programme on chlorinated organic microcontaminants (OVOC, the Dutch acronym for Chlorine Chain Follow-up Research Programme on Chlorinated Microcontaminants). This programme was commissioned by the Dutch government (Ministry of Housing, Spatial Planning and the Environment [VROM] and Ministry of Transport, Public Works and Water Management [V&W], Directorate General for Water Affairs), the Association of the Dutch Chemical Industry (VNCI) and the Netherlands Society for Nature and Environment (SNM). The current OVOC programme is the follow-up of an earlier research project that ended in 1995. This project included an assessment of environmental effects throughout the whole life cycle of chlorinated products and covered 99% of chlorine fluxes in the Netherlands.

OVOC reports

A series of 15 technical reports prepared during the project present detailed results of the different subprojects. These technical reports are included on the CD-ROM that is attached to the back cover of this report.

This report

The *abstract* provides a one-page overview of the set-up and main results of the OVOC programme. The first section of the *executive summary* describes the main overall conclusions of the study, against the background of the main research questions. The following sections contain the main findings from the separate studies on effluents, vent-gas emissions and products. Part 1 of the report describes the historical context of the project, the *scope and objectives*, and the selection of the potential emission sources. In Part 2, Chapters 3 and 4 present the *framework and design* of the experimental studies. Chapter 5 contains a presentation and discussion of the *main results* from the various subprojects. Part 3 presents the *synthesis and conclusions*. The synthesis in Chapter 6 addresses the main research questions and discusses inherent and remaining uncertainties in the final results. Final conclusions appear in Chapter 7. A list of abbreviations is included in the Appendix section (9.10).

Acknowledgements

More than 30 scientists at different laboratories and institutions were involved in the execution of this study. The authors gratefully acknowledge the willingness and kind cooperation of the companies who participated in the study. We further acknowledge the comments made by members of the steering committee and invited experts on the many draft versions of reports and during the fruitful and constructive technical and drafting workshops. Finally, we acknowledge the contributions that helpful colleagues have made to this study.

Abstract

This report presents the overall results of the Dutch research programme on emissions of chlorinated organic microcontaminants, i.e. unexpected chlorinated compounds with persistent, bioaccumulative and toxic (PBT) properties, originating from the Dutch chlorine chain. The main objectives of the programme were to assess whether and if so, to what extent the Dutch chlorine chain emits chlorinated organic microcontaminants with PBT properties and to contribute to the environmental impact assessment of these compounds.

The results of a prioritisation study formed the basis for the selection of industrial wastewater effluents, vent-gas incinerators, products from the chlorine chain and other sources (including an industrial cooling water system, a municipal wastewater treatment plant, and a municipal solid-waste incinerator). Samples of the selected sources and reference samples (Lake Marken surface water, tapwater, procedural blanks) were subjected to an analytical-chemical and biological-toxicological screening programme.

The toxicological assessment of wastewater effluents included both *in vivo* testing and *in vitro* bioassays for compounds with dioxin-like or estrogenic modes of action. Toxicity screening for vent-gas incinerator emissions and impurities in products was limited to the DR-CALUX *in vitro* bioassay. A variety of chemical methods was used to isolate fractions with potentially bioaccumulative compounds and for identification. Where relevant, the presence of dioxins (PCDD/Fs) was confirmed with appropriate methods.

The results of the present study indicate that chlorinated substances with potential PBT properties are present (and are probably being formed and emitted) as impurities in some production processes in the chlorine chain. Dioxins and some other chlorinated compounds with known or potential PBT properties, as well as tentatively identified and partially characterised chlorinated compounds were found to be present in various samples, especially in some product samples. Apparently because of improvements in production processes and the effectiveness of treatment systems that had been implemented for waste, atmospheric emissions and effluents, however, the estimated current emission levels identified in this study are much lower than known emission levels (of dioxins and some known PBTs), and they will not significantly increase environmental exposure levels on a national scale.

Emissions and effects are limited to a restricted number of production sites and products. All of the OVOC partners¹ therefore acknowledge that the results from this study indicate that there is no general 'chlorine-chain wide' problem with chlorinated organic microcontaminants with PBT properties that affects the entire chlorine chain in the Netherlands; only a limited number of possible problem cases were observed in this study.

¹ Dutch government (Ministry of Housing, Spatial Planning and the Environment [VROM] and Ministry of Transport, Public Works and Water Management [V&W], Directorate General for Water Affairs, the Association of the Dutch Chemical Industry (VNCI) and the Netherlands Society for Nature and Environment (SNM).

The unanticipated presence of dioxins and other chlorinated impurities in some products may be an area of concern that calls for further attention in current and future risk-assessment frameworks.

Key words: chlorine chain, PBT, effluents, dioxins (PCDD/Fs), TEQ, chlorinated products, toxicity tests: 8-day zebra fish-ELS, semi-chronic 22-hour Microtox test, DR-CALUX, Carp-hepatocyte EROD, VTG, MTT, analytical methods: RP-HPLC, GC-ECD/MSD; GC-NCI-MS, 2DGC-AED, HRGCMS.

Executive Summary

The present report contains a summary and synthesis of the results of the Dutch research programme on chlorinated organic microcontaminants (OVOC) commissioned by the Dutch government (Ministry of Housing, Spatial Planning and the Environment [VROM]; the Ministry of Transport, Public Works and Water Management [V&W], Directorate General for Water Affairs), the Association of the Dutch Chemical Industry (VNCI) and the Netherlands Society for Nature and Environment (SNM). The programme, executed between 1999 and 2004, was a follow-up action to the political, societal and scientific debate concerning the outcome of a national chlorine balance study conducted in the mid-1990s.

Objectives

The main objectives of the study were: 1) to assess whether and if so, to what extent unknown chlorinated compounds with PBT² properties (accepted or potentially persistent, bioaccumulative and/or toxic compounds) or groups of compounds with PBT properties are emitted from the Dutch chlorine chain and 2) to contribute to the assessment of the environmental impact of these emissions.

Methods

The results of a prioritisation study formed the basis for the selection of 16 industrial wastewater effluents, 3 vent-gas incinerators and 11 products from the chlorine chain. Other sources included a (petrochemical) cooling water installation, a municipal wastewater treatment plant (MWTP) and a municipal solid waste incinerator (MSWI). Samples of the selected sources and reference samples (water from Lake Marken, tapwater, blanks) were subjected to a programme of analytical-chemical and toxicological/biological screening.

The toxicological assessment of wastewater effluents included both *in vivo* testing and *in vitro* bioassays for compounds with dioxin-like or estrogenic modes of action. Toxicity screening for vent-gas incinerator emissions and impurities in products was limited to the DR-CALUX bioassay. A variety of chemical methods was used to isolate fractions with potentially bioaccumulative compounds and for identification. Where relevant, the presence of dioxins (PCDD/Fs) was confirmed with appropriate methods.

The study was partially exploratory. To the extent possible, the methodology made use of validated methods, but such methods were not always available for all of the sample matrices included in this study. All of the less-validated methods that were used had first been discussed – along with any possible limitations and uncertainties associated with these methods – with a wide range of scientists from academia and industry.

² In this report the term PBT refers to a wider group of substances than the generally accepted 12 POPs from the UN Stockholm Convention. Further information: Section 3.3 and Appendix 9.5.

I. General conclusions

The general picture that emerges from this study is that there is no general problem with chlorinated organic microcontaminants with PBT properties that affects the entire chlorine chain in the Netherlands. A number of PBTs and substances with potential PBT properties was present in varying degrees and composition within some chlorine chain processes. Possible PBTs, as well as PCDD/Fs, were observed in some products.

Emissions may occur as a result of product use, vent gases and a limited number of effluents. Apparently because of improvements in production processes and the effective management of waste, atmospheric emissions and effluents, however, the current level of these emissions is much lower than that of known emissions and will not significantly increase levels of environmental exposure on a national scale.

Nevertheless, a few potential, as yet unanticipated emissions of known PBTs or compounds with PBT properties (e.g., the possible emission of dioxins and chlorinated impurities from some products) may be an area of concern that calls for further attention in current and future risk-assessment frameworks.

Based on the results the following conclusions were drawn with respect to the main research questions:

a. Are PBTs or compounds with possible PBT properties being emitted?

- **Effluents.** Dioxins (PCDD/Fs) were determined in one selected wastewater effluent. In this effluent, dioxins were present and the estimated contribution of PCDD/Fs (< 1 pg WHO-TEQ/L) was less than 1% of the DR CALUX response (after acidic clean-up). No other PBTs or substances with possible PBT properties were identified in extracts of effluents responsive in the toxicity tests.
- **Vent gases.** The studies on atmospheric emissions included three industrial vent-gas installations and a municipal solid waste incinerator (MSWI); the latter as a reference for known non-chlorine-chain sources of dioxins (PCDD/Fs). Dioxins (PCDD/Fs) were found in all of the flue gases from all three of the vent-gas installations and from the MSWI. Moreover, a few other PBTs (e.g., hexachlorobenzene, pentachlorobenzene) and two unidentified chlorinated compounds were found in the flue gases of one or more installations.
- **Products.** Dioxins (PCDD/Fs) were found in three of the eleven products sampled in this study. Moreover, some other known PBTs (e.g., hexachlorobenzene and pentachlorobenzene) were tentatively identified³ in some products; pentachlorophenol was tentatively identified in one product. A substantial number of other chlorinated compounds were tentatively identified in various products. According to a preliminary classification using measured or QSAR-predicted properties, these compounds may have P and B characteristics. The positive finding of dioxins (PCDD/Fs) in chloroprene rubber was unanticipated: this product was not selected based on the prioritisation study, but on an ‘informed-guess’. Additionally, many highly chlorinated impurities, which could not be fully identified but some of which potentially had P and B properties, were observed in some prod-

³ Explanation of status of identification: see Section 4.6.

ucts. The total annual loads of chlorinated impurities in products were estimated based on national usage data (excluding on-site captive use; some consumption data were only rough estimates), and varied between <2 kg/yr (four products) and 20-200 kg/yr (chloroprene rubber).

b. Contribution to the assessment of environmental hazards and risks

- **Effluents.** None of the extracts of the aquatic effluents was responsive in the (OECD validated) *in vivo* zebra fish ELS test. Based on a comparison with other published data, it was concluded that the toxicity of effluents from the chlorine chain is not significantly different from non-chlorine-chain industrial effluents.
- **Vent gases.** The load of dioxins and/or dioxin-like compounds associated with the three vent-gas emissions is insignificant, compared to known dioxin emissions in the Netherlands.
- **Products.** Although some of the chlorinated compounds with PBT properties that were identified in products are potentially harmful to the environment, preliminary estimates of the total quantities of potential emissions on a national scale are low, compared to known emissions of chlorinated substances that OSPAR classifies as hazardous. It is unlikely that such emissions will significantly increase the level of environmental exposure to these compounds on a national scale.
- **Total loads of bioassay-based dioxin equivalents.** The total annual load of DR-CALUX-based bio-TEQs⁴ (after refined fractionation and/or acidic clean-up) was estimated at 4.4 g bio-TEQ/yr (effluents: 2.7 g, of which cooling water: 2.1 g and wastewater 0.6; products: <1.7 g; vent-gas treatment: 0.06 g). In a worst-case approach, it was assumed that the bioassay-based bio-TEQs determined in this study would be equivalent to the TEQs of dioxins and dioxin-like compounds (I-TEQs, WHO-TEQs). Even in this worst-case estimate, the total contribution from the chlorine chain and cooling water is limited (<6–11%), compared to values from national inventories of emissions of dioxins (PCDD/Fs) to water and air (40 - 80 g I-TEQ/yr). Compared to loads of other sources of PCDD/Fs (e.g., potential emissions to land and solid waste [150-1760 g I-TEQ/yr]) or sources for which no reliable quantitative data are available (e.g., remobilisation of historic emissions from sinks) and trans-boundary inputs (to both water and air), this contribution is even less. Based on this comparison, the results indicate that current emissions from the chlorine chain make only a minor contribution to national environmental exposure levels to dioxins and dioxin-like compounds.

c. Uncertainties

It is not possible to quantify the uncertainties in the final overall results of this very complex and partially explorative study, which included some recently developed methods. Neither the qualitative analysis of the methodological limitations and uncertainties involved in estimating annual loads, however, nor the application of worst-case approximations provide any indication that a more detailed and more comprehensive sampling design would have led to fundamentally different overall conclusions.

⁴ For an explanation of conventions used for expressing dioxin-equivalent concentrations determined with biological or chemical methods, see Section 3.3.

II. Detailed results: wastewater, vent-gas incineration and products

Wastewater studies

Sixteen industrial effluents originating from sites with chlorine chain activities in the Netherlands were sampled, as well as (for reference purposes) a cooling water system that uses hypochlorite as a biocide, the effluent of a municipal wastewater treatment plant (MWTP) and a body of natural surface water (Lake Marken). All samples were taken under normal operating conditions. For practical reasons, a tiered approach was chosen for analysing the aquatic effluents. The first steps of this approach consisted of separate screenings for toxicity (T), bioaccumulative potency (B) and the presence of chlorinated compounds. After selecting the most responsive effluents from the screening studies, the persistence (P) of the toxicity response, the attribution of responses to chlorine-chain activities and the identity of responsive compounds were studied

Extracts of the effluents were screened for the presence of chlorinated compounds (AOX, EOX, GC-ECD) and for the presence of a toxic response with both *in vivo* tests (e.g., the [OECD validated] 8-day zebra fish Early Life Stage test and chronic 22-hour Microtox test using luminescent bacteria) and *in vitro* tests with liver cells from carp (Carp-hep EROD, VTG, MTT) or rats (DR-CALUX test) to screen for the presence of dioxin-like or estrogenic effects.

Fractionation of sample extracts by reversed phase HPLC was performed to obtain fractions of different polarity or hydrophobicity with a less complex composition and to determine the extent to which the response (*in vitro* tests only) was related to potentially bioaccumulative compounds. Additional screening with GC-MS and GC-NCI-MS in responsive fractions was used to identify potentially responsive chlorinated compounds.

The presence of halogenated compounds was confirmed in nearly all of the effluent samples and in the cooling water sample, based on concentrations of EOX and AOX, as well as on the results of GC-ECD screening for extracts and fractions. Analytical results indicated that most of the chlorinated organics in the effluents was apparently either volatile or relatively polar ($\text{Log } K_{ow} < 2$), with decreasing amounts in fractions of increasing hydrophobicity.

Toxicological and biological testing revealed that none of the extracts of the aquatic effluents was responsive either in the *in vivo* zebra fish ELS test or in the *in vitro* Carp-hep tests for cytotoxicity or estrogenicity. A varying number of industrial effluents, the cooling water and MWTP samples were all responsive in one or more of the other tests (Microtox *in vivo* test, Carp-hep EROD and DR-CALUX *in vitro* assays) but not always the same ones.

A separate study compared the results of the *in vivo* tests on effluent extracts to the results of studies, carried out by RIZA, on whole effluent toxicity. The study concluded that chlorine-chain effluents do not differ from other industrial effluents in the Netherlands with respect to *in vivo* toxicity.

Response in the DR-CALUX test was concentrated in the medium and high hydrophobic fractions. All of the samples that were responsive in the carp-hep EROD tests were also responsive in the DR-CALUX test. Further detailed fractionation studies demonstrated that the DR-CALUX response was related to components with a $\text{Log } K_{ow} > 6$ or $\text{Log } K_{ow}$

>8 in some fractions. An acidic clean-up step was applied to minimise the contribution of responsive compounds that are neither dioxins nor can be classified as dioxin-like compounds (i.e., *transient inducers*, such as some PAHs). After this treatment, only two industrial effluents were still responsive (with 7 and 2 pg bio-TEQ/L, respectively), as was the cooling water sample (11 pg bio-TEQ/L). In the final responsive fraction, GC-MS or GC-NCI-MS screening failed to identify any chlorinated compounds.

A worst-case estimate of the annual loads of DR-CALUX activity possibly associated with dioxin-like compounds in the responsive effluents amounts to a total of 2.7 g bio-TEQ/y on a national scale (cooling water: 2.1 g; wastewater: 0.6 g). The worst-case estimate of the wastewater contribution (0.6 g bio-TEQ/yr) is higher than the known direct emissions of dioxins (PCDD/Fs) from industrial wastewater (0.04 - 0.17 g I-TEQ/yr) found in national emission inventories, possibly as a result of the worst-case assumptions that were made. Compared to the total emission of dioxins to water and air included in national inventories (approximately 40-80 g I-TEQ/y), the worst-case estimate of 2.7 g bio-TEQ/yr from cooling water and wastewater effluents would mean an additional contribution of 3-7 %.

Detailed in-plant studies were conducted at one production site with mixed chlorine-chain and non-chlorine-chain activities to determine the contribution of chlorine-chain activities to the response of the effluent. The results confirmed that the DR-CALUX response (after acidic clean-up) and the concentrations of chlorinated compounds originated mainly from the chlorine-chain activities. Although additional analysis confirmed the presence of dioxins (PCDD/Fs) in the final effluent sample (< 1 pg WHO-TEQ/L), less than 1% of the DR-CALUX response was attributable to this class of compounds. Identification studies with GC-MS and GC-AED revealed a large number of individual chlorinated substances (e.g., chloro- (di) propyl ethers) in the low Log K_{ow} fraction of wastewater from chlorine activities (0.5 mg/L). The corresponding medium Log K_{ow} fraction revealed a number of unidentified chlorinated compounds, but at much lower concentrations. No chlorinated compounds were observed above the limit of detection in the high Log K_{ow} fraction of the sample of chlorine-chain activities.

To screen for the persistence of the toxicity response in aquatic effluents, six samples from the screening phase were subjected to a biodegradation test (using surface water taken from the Eastern Scheldt as a reference), with the goal of simulating conditions in saline surface waters. The biodegradation test reduced the response for one out of four responsive samples in the Microtox test, possibly indicating the involvement of degradable compounds in the response of that sample. The results for the DR-CALUX response without acidic clean-up were inconclusive, due to an unexpected response in the reference surface water. After the application of acidic clean-up, only one sample was responsive in the DR-CALUX test following the biodegradation test. The effects of the biodegradation tests on the DR-CALUX response (after acidic clean-up) could not be evaluated in any of the samples, due to differences in detection limits before and after the tests.

Atmospheric emissions

The studies on atmospheric emissions were limited to three industrial vent-gas installations and a municipal waste incinerator that served as a reference for known non-chlorine chain sources of dioxins (PCDD/Fs). Sample extracts were subjected to toxicity screening with the DR-CALUX *in vitro* assay, chemical screening with GC-MS and PCDD/Fs analysis with HRGCMS.

The observed DR-CALUX responses were concentrated in fractions of high ($\text{Log } K_{ow} > 6$) and medium ($\text{Log } K_{ow} 4-6$) hydrophobicity. Contributions from analytically confirmed PCDD/Fs explained most (possibly as much as 80 to 90%) of the response after acidic clean-up for three of the four emissions. Only 22% of the response in one vent-gas installation was attributable to dioxins.

Identification studies revealed the presence of a few chlorinated compounds at levels between 0.1-80 $\mu\text{g}/\text{Nm}^3$: hexachlorobenzene (0.2-4 $\mu\text{g}/\text{Nm}^3$), pentachlorobenzene (2-7 $\mu\text{g}/\text{Nm}^3$) and two partially identified chlorinated compounds, one with a monochlorophenol fragment (2-80 $\mu\text{g}/\text{Nm}^3$) and the other a tetrachlorinated compound (0.1-1 $\mu\text{g}/\text{Nm}^3$). The load of DR-CALUX response (after acidic clean-up) associated with the three vent-gas emissions was 0.06 g bio-TEQ/y, which is considered insignificant relative to the levels of known dioxin emissions (approximately 40-80 g I-TEQ/y) to water and air in the Netherlands.

Products

Eleven selected commercial products were subjected to toxicity screening with the DR-CALUX bioassay and to chemical screening with GC-MS. Additional HRGCMS analysis of PCDD/Fs was conducted for the three most responsive samples.

DR-CALUX-based activity (after acidic clean-up) was observed at well-quantifiable levels in extracts of chloroprene rubber (CR) and, to a lesser extent, in hydrochloric acid (HCl) and trichloroethylene (TRI). Two other products, 1,2-dichloroethane (EDC) and perchloroethylene (PER), were responsive at levels close to the limit of detection. Additional analysis of dioxins in the three most responsive samples confirmed the presence of PCDD/Fs in these samples (0.3-90 pg WHO-TEQ/g); this could explain only between 5 and 21% of the observed DR-CALUX response, however. The remainder was due to unidentified responsive compounds, possibly including dioxin-like compounds.

Based on worst-case assumptions, the total load of DR-CALUX response (after acidic clean-up) associated with the commercial product flows is about 1.7 g bio-TEQ/y and is dominated (96 %) by the contribution of CR. Only a portion of this product-based load is ever emitted into the environment depending on the final fate of the products. The emission load of DR-CALUX-responsive compounds through product use is therefore of minor importance, compared to the known dioxin emissions to air and water (40-80 g I-TEQ/y).

The GC-MS screening studies demonstrated the presence of complex mixtures of impurities. Between three and seventeen chlorinated compounds (for each product) could be tentatively identified in eight of the eleven products, with an acceptable reliability at indicative concentration levels of between 10 and 100 mg/kg for CR (Soxhlet-extractable

compounds) and 10^{-5} -1 mg/kg for the seven other products. Further manual examination of unidentified components revealed the additional presence of multiple chlorinated compounds, especially in TRI, allylchloride (AC), monochloroacetic acid (MCA), and CR, with total concentrations ranging from between 0.1 and 1 mg/kg for MCA and TRI to between 10 and 100 mg/kg for CR. In many cases, homologous series of related compounds with varying degrees of chlorination were indicatively identified. With the exception of MCA, these series were partially attributable to the specific production process. Total annual loads of chlorinated impurities were estimated using national usage data (excluding on-site captive use; some consumption data were only rough estimates), and varied from less than 0.04 kg for two products (DCM and PER), <0.1-1.5 kg for TRI and HCl, 0.2 –2 kg for AC, <0.8-8 kg for EDC and <1-11 kg for MCA, up to between 20 and 200 kg for CR. These amounts can be considered as worst-case estimates for emission loads of possibly P and possibly B substances into the environment through the products route. Actual emission loads depend on consumption patterns, emission routes or waste-handling practices, and they may be much lower than these estimates. Because the production processes for some products on the Dutch market (e.g., TRI, PER, HCl) also differ significantly (in terms of impurity level and profile) from the specific product sources included in this study, a considerable level of uncertainty may be expected for extrapolations to the total national market.

Preliminary characterization of PB properties

A preliminary assessment of 45 chlorinated compounds, tentatively identified as impurities in products or present in vent-gas emissions, revealed that 22 of those compounds could be classified (following the OSPAR criteria) as potentially bioaccumulative ($\text{Log } K_{ow} >4$), and two could be classified as potentially very bioaccumulative ($\text{Log } K_{ow} >6$). Eleven compounds appear on existing priority lists of international environmental organisations. Because the volume of experimental data available for many compounds was expected to be insufficient for classifying persistency or toxicity, and because the systematic collection and review of such data would exceed the scope of the study, QSAR-based predictions were used as an initial, rough screening for persistency. Because of the high level of uncertainty involved, available toxicity-prediction programmes were not applied. Using preliminary (and uncertain) predictions of the classification of biodegradability with the BIOWIN programme of the US-EPA and the classification of the EU Interim PBT strategy, 25 of the 45 compounds were classified as probably persistent. Although several of the tentatively identified chlorinated compounds in the product extracts studied may be potentially bioaccumulative or persistent, and possibly toxic and harmful to the environment, the quantities involved are low, compared to known emissions for some of these compounds from other sources. Because of the low quantities involved, these compounds are unlikely to present any environmental risk on a national scale. Furthermore, some of the impurities that are present in some of the products may not be emitted to the environment, as these products are intermediates used in subsequent production processes.

Part 1 Introduction and Background

1. General introduction

The present report contains the results of a research programme on chlorinated organic micro contaminants (OVOC - Chlorine Chain Follow-up Research Programme on chlorinated micro contaminants), commissioned by the Dutch government (Ministry of Housing, Spatial Planning and the Environment [VROM] and Ministry of Transport, Public Works and Water Management [V&W, Directorate General for Water Issues]), the Association of the Dutch Chemical Industry (VNCI) and the Netherlands Society for Nature and Environment (SNM)⁵.

1.1 Context and framework

In 1995, the Netherlands Organisation for Applied Scientific Research (TNO) and the Centre of Environmental Science of the Leiden University (CML) presented their study on the chlorine chain (Tukker *et al.*, 1995). The design of that study included Substance Flow Analysis (SFA), Life Cycle Impact Assessment (LCIA) and Risk Analysis (RA). The study quantified 99 % of the chlorine fluxes in the Netherlands (see Figure 1.1). It also reviewed existing risk assessments for over 40 individual compounds, concluding that the risks associated with these (known) emissions were limited.

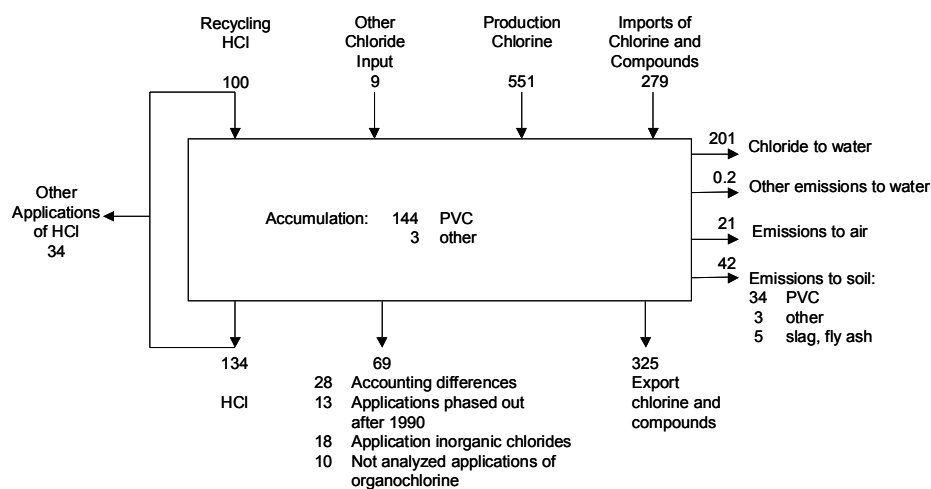


Figure 1.1 Summary of aggregated fluxes of chlorine in the Netherlands, 1990. Based on Tukker *et al.* (1995). Values are given in kt/year.

The study did not end the debate, partially because of the inherent limitations of the study and partially because of differences in the 'basic positions' or 'frames' of stakeholder coalitions in the toxicity debate (Tukker, 1998). The problem of 'chlorinated micropollutants' was a main unresolved issue identified by both the environmental movement and the peer review panel for the chlorine chain study.

⁵ Inception phase: Letter of Min VROM, dated 1 March 1999; subject DGM/SVS/99129326 zaaknr. 99230300; Phase-I: Letter of Min VROM, dated 30 September 1999; subject DGM/SVS/99217667 zaaknr. 99230300; Phase-II: Letter of Min VROM, dated 30 November 2000; subject DGM/SAS/200144640 zaaknr. 99230300.

Small amounts of unknown substances not considered in the emission inventory of the chlorine chain study could represent significant risks (e.g., should they have PBT properties, as do dioxins, for example).

As an illustration of potential knowledge gaps, studies analysing the occurrence of organochlorinated compounds in environmental media tend to succeed in identifying only a limited part of these compounds. As an example, the study of Loganathan et al. (1995) concerning Buffalo River (NY, USA) could attribute only between 10 and 20% of the total extractable organochlorine content ((EOCl) found in fish fat and sediments to such known (and now well-managed) chlorinated compounds as pesticides, PCB's and dioxins. In theory, the remaining, major part of the chlorinated organic compounds is attributable to four sources (see Figure 1.2):

1. Chlorine is reactive. There is considerable documentation about the presence of unexpected chlorinated by-products in various production processes. Once formed, these by-products might continue to be emitted unknowingly;
2. Relatively low-toxic chlorinated organic compounds, emitted in high volumes, could be converted into more toxic chlorine compounds by degradation processes⁶;
3. The compounds might consist of degradation products of the much higher anthropogenic emissions from the past;
4. They might consist of naturally produced chlorinated organic compounds.

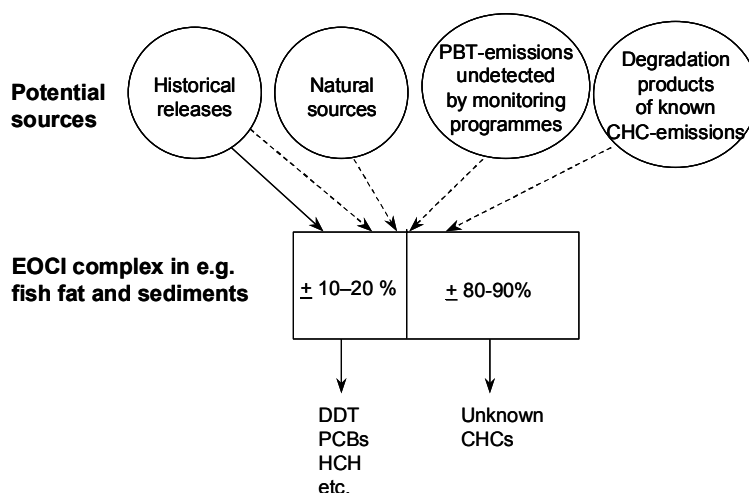


Figure 1.2 Potential sources of organochlorine compounds in the environment.

Following the finalisation of the chlorine chain study, the Minister for the Environment appointed a broadly composed committee (BITAC, Committee for the Implementation of Actions of the Chlorine Chain Study). One of the tasks of the BITAC was to formulate an approach to solving the knowledge gap concerning chlorinated micropollutants.

⁶ For example, there are indications that PERC, DCM or other compounds can be converted into such substances as phosgene or chloroacetic acid in the environment (Landner et al., 1995).

This resulted in a proposal for a further study, which would focus on analytical-chemical and ecotoxicological research to ascertain the relevance of the first source mentioned above (undetected PBT emissions)⁷. This subsequently formed the basis of the current OVOC study (Chlorine Chain Follow-up Research program on Chlorinated Organic Microcontaminants).

1.2 Scope and objectives

The objectives of the OVOC study were to provide answers to the main questions raised and agreed upon by the BITAC⁸ committee (with representatives from government, industry, trade unions and environmental NGOs) in 1997: Does the problem of chlorinated microcontaminants (still) exist? If so, what is the extent of the problem? Which PBTs (or groups of PBTs) are emitted from the chlorine chain into the environment from point or non-point (diffuse) sources?

In this study, the term PBT refers to (chlorinated) substances with proven or potential persistency (P), bioaccumulation (B) and toxicity (T) characteristics, including generally accepted PBTs (within OSPAR, UNEP), as well as substances meeting specific criteria for P, B and/or T properties.

During the preparatory consultations in 1998, these objectives were further operationalised and extended to:

- Assess whether and to what extent PBTs or groups of PBTs are emitted from the chlorine chain into the environment;
- Contribute to the assessment of the environmental impact of these PBTs.

The OVOC project had a total duration of more than four years and consisted of supporting background studies and experimental screening studies using integrated chemical and biological methods. The research team, supervising committee, and invited experts held technical workshops to evaluate and review intermediate results and to select and prioritise samples for additional studies. The table below indicates the different phases and timetable of the programme (Table 1.1). Invited experts and workshop participants reviewed the technical reports of the different experimental and background studies. The technical reports and workshop reports are included in the Appendix CD-ROM, distributed together with this report.

A full description of objectives, scope and backgrounds for the choices made is provided in the original programme proposal (Van Hattum et al., 1998) and the work plan for the Phase-II main studies (Van Hattum et al., 2000) both included in the Appendix CD. Appendix section 9.3 describes the content of this CD-ROM.

⁷ The other routes would be covered only by relatively limited literature studies.

⁸ Minutes BITAC meetings: September 25th 1997 and December 9th 1997.

Table 1.1 Organisation and timetable of the OVOC project. R1-R15: technical reports included on the Annex-CD (see Annex 9.3 for content of CD).

Phase	Period	Experimental studies	Background studies	Workshops	
0	1999	<i>Inception phase</i>			
1	2000	<u>Pilot studies.</u> <ul style="list-style-type: none"> • Optimisation of chemical and biological methods (R1) 	<u>Supporting literature studies</u> <ul style="list-style-type: none"> • Unknown organochlorines in the environment (R2) • Degradation products (R3) 		
			<u>Prioritisation study</u> <ul style="list-style-type: none"> • Emissions and priority setting (R4-5) 	W-1	
2	2001	<u>Main studies</u> <ul style="list-style-type: none"> • Aquatic effluents (R6-9) • Atmospheric emissions (R10) 		W-2	
	2003	<ul style="list-style-type: none"> • Products (R11) 			
		<u>Additional studies</u> <ul style="list-style-type: none"> • Validation of clean-up (R12) • Effluent toxicity comparison (R13) 	<ul style="list-style-type: none"> • Emission reduction measures (R14) • Dioxin emissions (R15) 	W-3	
3	2004	<i>Synthesis and final reporting</i>			W-4

2. Chlorinated microcontaminants: priorities and other background studies

As indicated in Section 1.1, the philosophy of the OVOC study was to use analytical-chemical and biological/toxicological tests as a basis for investigating potential sources of chlorinated micropollutants, in relation to the production and use of chlorine in the economic system. The measurement program of the study would concentrate on end-of-pipe emissions to water and air, and on contamination of products.

To facilitate the execution of the analytical work in the main phase and the interpretation of results, we conducted a number of literature studies.

a) Studies that determined priorities for analytical research

In the Netherlands, there are several dozens of plants using chlorine and/or chlorinated substances emitting chlorinated substances to air and water. Furthermore, several hundreds of chlorinated products are on the Dutch market. The budget did not allow for analysis of all these potential targets, thus necessitating a procedure for setting priorities.

b) Studies facilitating interpretation

Though the OVOC study has not as aim to solve the relative contributions to the unidentified chlorinated compounds in environmental samples of all four sources in Figure 1.2, some literature work was done that could help to put any results from the OVOC monitoring programme in perspective. It concerns:

- A study into the likelihood and potential relevance that known chlorinated hydrocarbons emitted degrade to substances with PBT properties;
- A study on lesser-known chlorinated PBTs, as reported from field and monitoring studies;
- A study into the historical and present Dutch emissions of dioxins, which –with all caution – might help to put any emissions of bio-TEQ TCDD measured into perspective.

Section 2.1 first briefly introduces the Dutch chlorine chain and discusses the approach followed in the priority setting procedure. Section 2.2 then discusses the results of the studies facilitating interpretation.

2.1 Priority setting and selection of sources

Chlorine processes considered

In view of the formation of possible PBTs, it is possible to distinguish various types of processes:

1. Processes in the chemical industry, in which chlorine is used as a building block in chemical reactions. Here, unwanted by-products may arise that could be emitted either to air or to water;
2. Processes, where chlorinated products (e.g., solvents) are used but not transformed into other compounds;

3. Processes, where active chlorine is used as a means to prevent algae and bacteria growth (e.g., hypochlorite)⁹;
4. Processes in which chlorine plays no main role, but is present in inorganic or other forms, and may thus be converted into more harmful compounds.

Information from the chlorine-chain study formed the basis for creating a long list of 63 chlorine-chain processes and products. This list appears in Appendix 9.1.1.

Criteria for priority setting

In the priority setting, a number of common elements can be used to rank processes/products on their chance that they may contribute significantly to a chlorinated micropollutant problem:

1. The chlorine throughput in a process, or the volume of product use. In such cases, even very small percentages of unintended chlorinated contaminants emitted from the process or present in the product may lead to significant micropollutant problems.
2. The volume of emissions of known chlorinated substances. In addition, here, even small percentages of chlorinated by-products and/or emissions of unintended chlorinated compounds may lead to a significant micropollutant problem.
3. The chance on the formation on by-products. In literature, in general the following criteria are mentioned (see e.g., the dioxin reviews of Bremmer, 1994, and Fiedler, 1994):
 - The presence of active chlorine;
 - The availability of precursors that, together with active chlorine, may easily form 'complex' compounds¹⁰;
 - The presence of a catalyst or UV-light;
 - High temperatures;
 - For water emissions: inconsistencies or a mismatch between the volume of known emitted components (as derived from measurements of individual compounds) and the total discharges of EOX or AOX;
 - Indications from previous investigations or literature concerning the formation of by-products.

We used several literature sources in the valuation of these criteria, filling information gaps by expert judgement.

⁹ Paper and pulp bleaching, another important application, is virtually absent in the Netherlands.

¹⁰ For instance, if organic components with ring structures are already available in raw materials, there is a higher chance that aromatic chlorines will form. For instance, Fiedler (1994) claims that the chance on finding dioxins as a by-product in production of the following compounds decreases in the following order: chlorophenoles > chlorobenzenes > aliphatic organochlorines > inorganic chlorine compounds.

Results of priority setting

In interaction with the BOVOC Steering Committee, the criteria described above were used to define a selection of priorities that consisted of 16 wastewater emissions, 4 air emissions and 11 products. In addition, the list included two ‘wild card’ products (i.e., products that were not included in the priority-setting procedure but that were proposed by environmental NGOs based on an ‘informed guess’). The details of this process appear in Appendix 9.1, and the resulting list appears in Table 2.1 (emission sources) and Table 2.2 (products), along with the sample codes for emissions of wastewater and air emissions¹¹.

These codes were used during the execution of the experimental work and were known only by a small group of researchers. Individual companies received information about their own codes during the discussion of the intermediate results and draft reports. This final Technical Report finally discloses the codes to the BOVOC steering committee as well.

2.2 Studies facilitating interpretation of experimental results

Less-known chlorinated compounds reported from literature

Most of the information on chlorinated PBT compounds in the literature is confined to well known compounds such as PCBs, dioxins, DDT and other first generation chlorinated pesticides, present on international priority lists. Some of these compounds are sometimes known as the ‘dirty dozen’. Klein et al. (2001a) conducted a literature study on lesser-known chlorinated compounds detected in various environmental compartments in relation to potential anthropogenic sources, which could be useful for some of the initially scheduled field studies. We compiled a list (of approximately 250 compounds, summarized in Appendix 9.2, Table 9.2.1), including many different classes of halogenated compounds, ranging from simple aliphatic compounds to complicated multi-heterocyclic aromatic compounds.

In many cases, the possible sources of these compounds were unknown. Some identified sources are the production processes of polymers, dyes, flame retardants, plasticisers and textiles, wastewater disinfection, waste incineration and the production of pulp and paper. Because sampling and screening of sediment and fish samples that were scheduled to take place at the end of the project were not executed, due to the limited success of the effluent identification studies, the results of the literature study were not used further in the project.

¹¹ The mass flows in the Dutch chlorine chain study indicate that the products listed in Table 2.2. account for around 70% of the flow of the organochlorine compounds produced in the Netherlands (excluding PVC; this material had already been analysed thoroughly in other studies and was not deemed a priority product).

Table 2.1 Aquatic and atmospheric emission sources, prioritised for OVOC study.

Company name	Main processes ^{1,2)}	Non-chlorine contribution ³	Water	Air
<i>Industrial chlorine processes</i>				
Akzo Nobel, Botlek	Chlorine production (2)		MAB	
Shin-Etsu, formerly Rovin, Nufarm, formerly Akzo Nobel, Botlek	EDC/VCM production (3) Production MCPA/MCPP (4)		MBC	270-04
Resolution Products, formerly Shell Pernis, Shin-Etsu Pernis formerly Rovin	ECH and AC production (5) PVC Production (6)	yes	MCD	270-02
Akzo Nobel Hengelo,	Chlorine production MCA production (8)	yes	MDE ⁴	
Akzo Nobel Farmsum/Delfzijl	Production chlorine, solvents		MEF	
Teijin Twaron BV, formerly Aramid Products, Delfzijl (part Acordis)	Production of aramide and precursors (10)		MFG	270-05
Noveon, formerly BF Goodrich, Delfzijl	Production C-PVC (12)		MGH	
Dow Benelux Terneuzen	Production ethylene amines (11)	yes	MIJ	
Du Pont de Nemours Dordrecht ⁵	Production HCFCs and Teflon (15)		MJK	
			MKL	
Crompton Europe BV (formerly Uniroyal Amsterdam)	Production insecticides (17)		MLM	
Sappi, Maastricht	Paper recycling	yes	MNO	
Hercules Zwijndrecht	Production of ECH-derivates (19)	yes	MOP	
Kerr-McGee pigments, formerly Kemira pigments, Rotterdam	Titanium dioxide production		MPQ	
Akzo Nobel Kleefsewaard	CMC production (18)	yes	MQR	
Avebe, Veendam	Production modified starch (18)	yes	MRS	
Atofina, formerly Elf Atochem Vlissingen ⁶	Production of organotin (17)	n.a.		
Givaudan Roure, formerly Tastemaker ⁷	Production with EDC (20), ceased per end of 2000	n.a.		

Company name	Main processes ^{1,2)}	Non-chlorine contribution ³	Water	Air
<i>Non-industrial process applications</i>				
AVI Amsterdam	Municipal waste incineration	n.a.		270-01
Oil refinery, Europoort	Chlorine in cooling circuits		MST ⁸	
Cooling water outlet				
<i>Processes suggested by Steering committee (BOVOC)</i>				
MWTP Sleen	Municipal /non-industrial	n.a.	MMN	
Lake Marken	Reference, surface water	n.a.	BTU	
Tap water Lelystad	Reference sample	n.a.	BUV	

¹ Chlorine-chain processes as referred to in priority setting procedure (Appendix 9.1);

² Between brackets: chapter in background document Tukker (2000b);

³ Refers to non-chlorine production processes contributing to effluent as sampled (n.a. = not applicable);

⁴ Effluent sampled after treatment in the municipal wastewater treatment plant of Hengelo;

⁵ Discharge via 2 installations: MJK acid stream; MKL basic stream;

⁶ Not included in study (see Section 4.2);

⁷ Production with EDC had ceased in 2001;

⁸ Additional sample taken of inlet make-up water (code BST).

Table 2.2 Products prioritised in OVOC study.

Product	Supplier
<i>Products produced in NL</i>	
1,2-dichloroethane (EDC)	Shin Etsu, Rotterdam
allylchloride (AC)	Resolution, Rotterdam
monochloroacetic acid (MCA)	AKZO Nobel, Hengelo
dichloromethane (DCM)	AKZO Nobel, Delfzijl
hydrochloric acid (HCl)	AKZO Nobel, Delfzijl
<i>Products not produced in NL</i>	
perchloroethylene (PER)	Solvay, Tavaux, France
trichloroethylene (TRI)	Solvay, Tavaux, France
1,4-dichlorobenzene	Bayer, Leverkusen, Germany
chloroparaffins C ₁₄₋₁₇	Ineos, Runcorn, UK
<i>'Wild cards' suggested by BOVOC</i>	
chloroprene rubber	Polycomp, Vorden
triclosan	Ciba, Basel, Switzerland

Role of degradation products

Degradation products of high-volume substances, which are emitted to air and transformed in the environment, are likely to end up in other environmental compartments. The degradation pathways and transformation products of a number of relevant high emissions of chlorinated compounds were identified (Klein et al., 2001b). The significance of specific degradation pathways may depend on the initial emission compartment, on the type of the emission (free compound or adsorbed to particles) and on such environmental conditions as temperature, intensity and the availability of solar radiation and of micro-organisms. The degradation products of low-emission compounds, including a large number of emissions to surface water, were not studied. Table 9.2.2 in Appendix 9.2 summarises the results).

Emissions of dioxins

In order to provide a framework for evaluating the estimated annual loads of bioassay-based dioxin equivalents in the experimental studies, a critical review was made of historical and existing data on emissions of dioxins in the Netherlands (De Koning et al., 2004). The most recent national dioxin balance (for 1999), as reported in national annual environmental assessment reports (RIVM, 1999, 2002) was supplemented with data for emissions to water from the REVIEW database of RIZA, and compared with inventories made in recent European studies (Quaß et al., 2004; Pacyna et al., 2003; Wenborn et al., 1999). Table 2.3 summarises the main results.

The category 'contributions to land' in the European inventories, includes the disposal of solid waste and application of fly ash and bottom ash in road construction materials and various other categories, and explicitly does not reflect actual emissions to soil. Only relatively old estimates are available. The recent Dutch inventories do not consider this category in detail.

Table 2.3 Estimated annual emissions of dioxins (PCDD/Fs in g I-TEQ/yr) in the Netherlands from recent national and European emission inventory studies.

Source category	National inventories 1999		Recent studies ⁽³⁾ and data, reviewed in de Koning (2004)	
	RIVM ⁽¹⁾ : air RIZA ⁽²⁾ : water g I-TEQ/yr		year	g I-TEQ/yr
<i>Waste management</i>				
• air	4		2000	10
• water	0.65		2000	0.024
• land			1994	127-822
<i>Industrial</i>				
• air	3		2000	43-58
• water	0.17		2000	0.04
• land			1994	17-170
<i>Non-industrial</i>				
• air	28		2000	13-14
• water	0.4-2		2000	0
• land			1994	2.3-768
Total, including land emissions				220-1840
Total, excluding land emissions	37-38			66 – 82

¹ RIVM (1999, 2002); ² Compiled from the review database of RIZA; ³ emissions to air: Quass et al. (2004); emissions to land: Wenborn et al. (1999), water: recent data in REVIEW database summarized in de Koning et al. (2004).

In the European studies, recent estimates for atmospheric emissions were approximately twice as high as were the values used in the RIVM inventory. This result was partially due to differences in the time periods considered and partially due to higher estimates in the European studies for sintering processes, accidental fires, illegal waste burning and domestic waste burning. Additionally, differences among the inventories may result from differences in estimation methods and aggregation levels. The study confirmed the realisation of drastic reductions in emissions to air, water and soil through the recycling of solid waste, especially during the last 20 years (for atmospheric emissions, see Figure 2.1).

Emissions to air for the specific source category *chemical production* (SNAP code 0405) are available for 1991 and were in the order of 0.5 g I-TEQ.yr⁻¹ (Bremmer et al., 1993). Water emissions in 1991 from chemical production were in the order of 0.5 g I-TEQ.yr⁻¹. More recent inventories provide only aggregated data and contain no individual data for the chemical production category. Comprehensive studies covering all potential water emissions have not been conducted in the Netherlands since 1991. Current efforts to monitor aquatic emissions of dioxins focus on the most significant sources. An analysis of the RIZA REVIEW database revealed that, in general, water emissions from these selected sources have decreased considerably during the last decade.

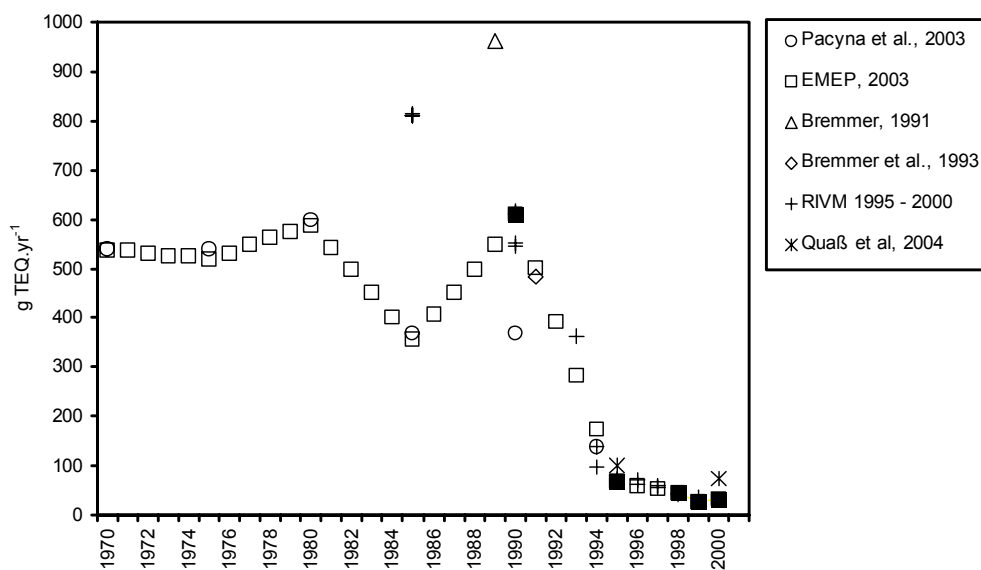


Figure 2.1 Total PCDD/PCDF emissions to air in the Netherlands (1970-2000), obtained from several data sources and expressed in g I-TEQ/yr.

Note: The RIVM 1995-2000 data represent the data published in the yearly (Milieubalans) reports from 1995 to 2000. For the EMEP data (EMEP, 2003), the solid markers (■) represent official data submitted to EMEP. National studies: Bremmer (1991), Bremmer et al. (1993), RIVM (1999, 2002). European studies: Pacyna et al. (2003), Quaß et al., 2004, EMEP (2003).

Part 2 Experimental Studies

3. Introduction

3.1 Objectives of the OVOC project

The main task of the experimental studies was to detect the presence of unanticipated chlorinated compounds with PBT properties in aquatic effluents, atmospheric emissions and technical products from the chlorine chain. Existing approaches for classification of PBT properties, as e.g., agreed within OSPAR or EU-REACH, are restricted to individual compounds or well defined compound classes, and are not suitable for the classification of properties and interactions of complex mixtures as in the OVOC project.

At the start of the project, an integrated framework of chemical and biological screening methods was designed for the aquatic effluents, in order to focus on relevant properties and compounds (see Figure 3.1). The target compounds of the study are especially those chlorinated substances, which can induce toxic effects (T) in higher organisms, due to bioaccumulation (B) in food webs, and their persistence (P) in the environment. This approach was tested in a pilot-study during Phase 1 (Belfroid *et al.*, 2000) and further adapted according to an evaluation conducted during the first technical workshop in May 2000.

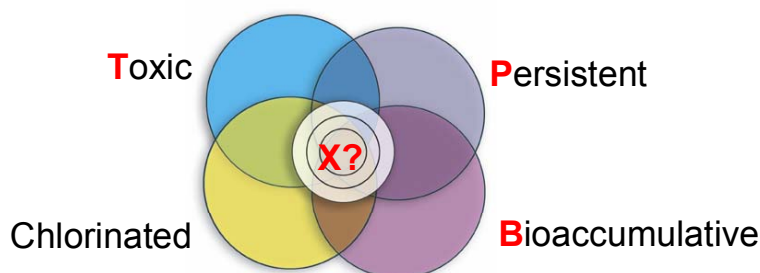


Figure 3.1 Conceptual scheme of target compounds in the present study.

Starting from the extensive groundwork on emissions from the chlorine chain as performed by the Chlorine Chain Study (Tukker *et al.*, 1995), the aim of the OVOC study was to focus on unknown and/or unanticipated emissions of chlorinated PBT substances.

The search strategy therefore is necessarily very broad, looking for known PBTs at unanticipated places as well as yet-unknown PBTs or PBT effects at more familiar places. With respect to the required chlorinated character of target compounds, non-chlorinated PBTs that are strictly linked to the chlorine chain were also considered as targets in this study.

The implementation of this broad search strategy involved the use of screening analytical techniques (GCMS, gas chromatography with mass spectrometric detection) and bioassays (screening for general or specific toxicity). It is this screening character, aiming not to overlook possibly relevant substances or effects, that should be born in mind when interpreting the results of the OVOC study. Substances or effects identified with the screening methodology must be considered as possible hits.

Additionally, dedicated analytical techniques (e.g., high resolution GCMS, HRGCMS, for dioxin analysis) are then required to corroborate the presence of the substance or effect under consideration. Wherever this corroboration was not been performed, the results should be considered to *indicate* the presence of a substance or an effect, which theoretically might also be a false positive result. Section 4.4 considers this issue in more detail for the *in vitro* assays.

Figure 3.2 schematically gives the major approach that OVOC relied upon. Identification of substances and measurements of toxicological effects formed the two pillars of OVOC studies in wastewater. Ideally, if enough data are available, the toxicological effects observed in emissions can be linked to the substances identified. This toxicity identification concept (commonly referred to as Toxicity Identification and Evaluation: TIE) was one of the leading principles in the OVOC design. However, due to the limitations of the methods used, and the complex composition of the emissions samples, the full TIE scheme could not be realised. Especially the identification of substances gave very limited results. Therefore, estimated emission loads of chlorinated microcontaminants with known or potential PBT properties are used as input for the assessment of environmental hazards, as are worst-case estimates of loads of toxicity equivalents.

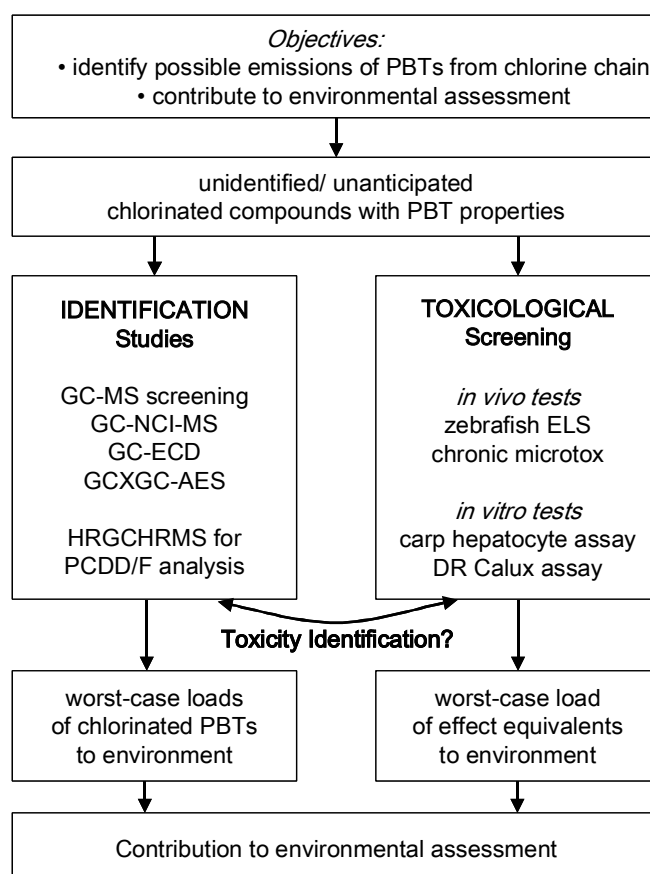


Figure 3.2 General approach of OVOC study: identification and toxicity screening.

3.2 Selection of toxicity-screening tests

For the classification of toxicity in the present study, we applied both *in vivo* tests (with whole organisms) for general toxic effects (mortality, growth, development), and sensitive *in vitro* tests with liver cells to screen for specific toxicity (dioxin-like toxicity, estrogenicity).

The results of responsive *in vivo* tests can be interpreted as a direct effect on test organisms at specific concentration levels, and can be used for input in environmental hazard and risk assessments. The outcome of the *in vitro* tests may indicate the exposure to specific compounds and the induction of specific effects (e.g., induction of genes or enzymes) at the cellular level, which do not necessarily have to lead to effects at the organismal level. A great number of repair mechanisms or adaptation effects may prevent this potential effect from becoming a real effect in the environment. We therefore treat the outcome as an indication of potential toxicity, possibly at higher levels in the food chain.

The *in vitro* tests applied were highly sensitive and specific for the types of toxicity selected (dioxin-like toxicity, endocrine disruption), which are relevant for organisms at higher levels in the food chain, and at least partly match with the profile of the target (PBT) compounds indicated in Figure 3.1. Although trivial, it should be realised, that there are no standard *in vivo* tests specific for chlorinated compounds, and that for most general toxicity mechanisms (narcosis, neurotoxicity, immunotoxicity) chlorinated compounds do not seem to be more responsive than halogenated compounds. For the dioxin-like toxicity syndrome, it is known that some chlorinated or brominated aromatic compounds are much more responsive than non-halogenated compounds, which was one of the reasons to select the specific *in vitro* tests for potential dioxin-like toxicity. In addition, in the societal debate on chlorine emissions of dioxins have often drawn specific attention.

Another important consideration for the selection of the tests was that they had to fulfil operational criteria with respect to sensitivity, reproducibility, sufficient level of validation, representation of different trophic levels, as well as the condition that the total sample quantity needed would be within practical limits.

The two *in vivo* tests selected were the (OECD-212 standard) zebra fish embryo (8-day) early life stage test (ELS), and a chronic version of the well-known MicrotoxTM test with marine-luminescent bacteria. For the *in vitro* tests on dioxin-like toxicity, two novel and sensitive tests were selected: the DR-CALUX assay, based on a reconstructed mammalian celline, and the Carp-hep EROD test based on a liver celline of feral carp. The (anti) estrogenic potency of samples was evaluated based on the measurement of the induction of vitellogenin (VTG) in the Carp-hep celline.

The application of widely used and – especially in the case of the zebra-fish ELS test – well validated tests for wastewater allows comparison of the results with related studies. The application of the *in vitro* tests to wastewater is relatively new, and neither test had been validated for the application to wastewater prior to the OVOC study. The advantage of using the combined approach is that it provides new information regarding specific toxicity (*in vitro* assays), while still keeping the *in vivo* tests as a reference for more general direct toxic effects. Note that any possible relation found between the *in vivo* and *in*

in vitro results applies only to specific samples, and cannot be generalised to effluents or classes of substances as a group. In addition, concurrence of *in vitro* and *in vivo* effects does not necessarily imply a cause-effect relation.

3.3 Criteria and screening methods for PBT characteristics

The attention for PBT substances originates from the insight that these substances can accumulate in the food chain, giving rise to toxic effects in higher organisms (e.g., water birds, seals, polar bears), even in such remote places as the North Pole. In order for PBTs to be considered as an environmental hazard, the following properties are relevant:

1. Transport over long distances: this implies that the PBTs should be neither biodegradable nor prone to hydrolysis (in water) or photolysis (in the case of atmospheric transport).
2. Accumulation in organisms and food webs: bioaccumulation (B) of organic compounds depends, strongly on the hydrophobicity of substances, the bioavailability, and biotransformation.
3. Limited biotransformation: PBTs must be sufficiently resistant to metabolic detoxification processes in the organism.
4. Expression of toxicity (T) in organisms.

Persistence (P) refers to the ability of a substance to withstand processes 1 and 3.

The term PBT is used in several contexts. First, it can refer to the persistent organic pollutants (POPs) that lead to adverse food-chain effects in the environment. The UN Stockholm Convention gives a list of twelve substances ('dirty dozen') that are widely recognised as such POPs. The term PBTs can also be used in a screening sense, however, indicating those substances that could *possibly* induce food chain effects. For example, the OSPAR Dyanmec mechanism involves criteria for P, B and T to screen for substances that may be environmentally hazardous. Substances that pass the screening are further *analysed* to assess the actual risk they pose to the environment. Substances exceeding certain risk levels are then included in the list for priority action.

In recent years, several international environmental policy frameworks have drawn up criteria for PBT substances. Although these criteria do not strictly coincide, there is a general tendency towards integration. Because of differences in perspective and aim (emission control vs. substance management), each list will adopt its own level of precaution in defining screening criteria for substances. Appendix 9.5 presents an overview of the PBT classification criteria currently adopted in the Oslo-Paris conventions (OSPAR), the EU Water Framework Directive (WFD), the Dutch strategy for management of substances (SOMS), the new EU strategy on substance management (REACH) and the UNEP strategic approach to international chemicals management (SAICM).

The lists of (priority) substances contained in the different conventions and assessment frameworks aim to name those substances about which there is consensus that they pose actual risks to man or the environment. Due to the limited knowledge available on hazardous properties of the many substances used in society, it is generally acknowledged that these lists are not comprehensive. Within many frameworks, therefore, screening methods exist (or are under development) that use general criteria to indicate whether unlisted substances have possible PBT characteristics. By their screening nature,

these criteria aim not to overlook possible PBT substances, accepting the possibility that PBT characteristics are attributed to substances in error.

Within the OVOC project, PBTs are referred to in a screening sense, implying those substances that, after more extensive analysis, may be recognised as actually leading to food-chain effects. Therefore, the following criteria for P, B and T were used:

- **Persistency:** the biodegradation test for aquatic effluents (see Section 4.7) was adopted to assess the persistency of (toxic) effects. This test was not used to describe persistency of individual substances. For the assessment of persistency of individually identified substances, the screening method proposed for the EU-REACH interim PBT strategy was adopted.
- **Bioaccumulation:** the Log K_{ow} value of extracts or substances is used to assess the potential bioaccumulation of substances or effects. Values above 4 are interpreted as indicating potential bioaccumulative substances, and values over 6 indicate potentially very bioaccumulative substances.
- **Toxicity:** extracts were tested in several *in vivo* and *in vitro* toxicity tests. This renders information on toxicity of the mixtures as present in the extracts. Toxicity was not assessed on the level of individual substances.

The criteria as given above are used in this study only to provide a first characterisation of substances and effects. They do not aim to give a final assessment of individual substances or emissions. Actual P, B and T criteria have been applied in Sections 5.5 and 5.7.

3.4 Dioxin-like compounds and biological/chemical assessment methods

Because the *in vitro* tests for dioxin-like toxicity were the most responsive in the OVOC project, and because an understanding of some of the background characteristics of this class of compounds can facilitate the proper interpretation of the results, this section provides additional information.

Dioxins

Polychlorinated dibenzodioxins (PCDDs) and dibenzofurans (PCDFs), commonly known as **dioxins**, are persistent organic pollutants (POPs) that are typically present in mixtures (varying in composition) of 210 possible congeners, of which 17 belong to the most toxic group. The 2,3,7,8-tetrachlorodibenzodioxin congener (2,3,7,8-TCDD) is the most toxic. In the context of *in vivo* studies, the toxic congeners exhibit a multiple combination of responses typical for dioxins (e.g., wasting syndrome, which is a decreased ability to retain or utilize the energy from ingested calories, chloracne, immunosuppression, hepatotoxicity, teratogenicity etc). Each congener of dioxins exhibits a different level of toxicity. In order to be able to sum up the toxicity of the different congeners, the concept of toxic equivalence factors (TEFs) was introduced to facilitate risk assessment and regulatory control. The toxicity of each toxic congener is expressed relative to 2,3,7,8-TCDD, the most potent congener.

Dioxin-like compounds

Various other polyhalogenated aromatic compounds have a mode of action similar to that of the toxic dioxins, and they are usually addressed as **dioxin-like compounds**¹². These compounds include some co-planar polychlorinated biphenyls (PCBs), some polychlorinated naphthalenes (PCNs) and some brominated flame retardants (polybrominated biphenyls, PBBs; polybrominated diphenylethers, PBDEs). At the subcellular level, all polyhalogenated dioxin-like compounds are associated with strong binding to and continuous activation of the arylhydrocarbon receptor (AhR) over prolonged periods of time. Most of the known dioxin-like compounds are resistant to biodegradation or metabolism, and may exhibit bioaccumulation and transfer in food webs, resulting in exposure of top predators such as marine mammals and piscivorous birds. Some of the dioxin-like compounds have been classified as POPs and PBTs in current regulatory and assessment frameworks. The WHO Working Group on dioxins (Ahlborg et al., 1994; Van den Berg et al., 1998) uses the following criteria to define a compound as dioxin-like, for which the derivation of a TEF value should be considered: (1) a compound that shares certain structural properties with the PCDD/Fs, (2) a compound must bind to the arylhydrocarbon (Ah) receptor, (3) a compound must elicit Ah receptor-mediated biochemical and toxic responses and (4) a compound must be persistent and accumulate in the food chain.

TEFs and TEQs

Dioxins have traditionally been measured with high-resolution gas chromatography in combination with high-resolution mass spectrometry (HRGCMS). Usually, only the 17 toxic congeners are measured. The chemically measured concentrations are multiplied by their respective TEFs to obtain a total 2,3,7,8-TCDD equivalent concentration (TEQ). The TEFs are based on acute toxicity values from *in vivo* and *in vitro* studies and were assigned by special international expert committees. To date, most studies have used one of two schemes. The older scheme involves the use of TEFs established by a NATO/CCMS Working Group on Dioxins and Related Compounds and International Toxicity Equivalency Factors (I-TEF; NATO, 1988), and is restricted to the 17 PCDD/F congeners. The most recent scheme established by a WHO/IPCS working group, who re-evaluated the I-TEFs and established a new scheme (UNEP, 2001; Van den Berg et al., 1998) providing TEF values (usually indicated as WHO TEFs) for 17 PCDD/Fs and for 12 dioxin-like coplanar PCBs. The latest EU Council regulation (EC No 2375/2001) on maximum levels of dioxins and dioxin-like PCB in food products follows the WHO-based system of TEFs. Appendix 9.4 (Table 9.4.2) indicates the I-TEF and WHO-TEF values. Current insights in the relative toxic potencies of other polyhalogenated dioxin-like compounds (Giesy et al., 2002, Behnisch et al., 2003) indicate that the proper assessment of the exposure to complex mixtures of all compounds with a dioxin-like mode of action requires future extension of the TEF system to these other dioxin-like compounds.

¹² Some studies treat PCDFs as a subgroup of the category of dioxin-like compounds. This study follows the conventions of most regulatory studies.

Bio-analytical screening

During the last decade, various bio-analytical screening methods (bioassays) have become available for detecting the presence of dioxins and dioxin-like compounds in environmental samples (Behnisch et al., 2001). They have been proposed as pre-screening tools for the labor- and cost-intensive chemical HRGCMS methods. All of these bioassays are based on the common mechanism of toxicity of dioxins and related compounds, which requires initial binding to the Ah receptor. Ah-receptor activation leads to the induction of many genes, including the enzyme cytochrome P450 1A, which can be detected using the EROD assay, as was done in the Carp Hepatocyte EROD assay. The DR-CALUX assay, as applied in the OVOC project, is based on the activation of a reporter gene (fire fly luciferase) that has been built into a genetically modified H4IIE rat hepatoma cell-line (Sanderson et al., 1996, Murk et al., 1996). Upon exposure to dioxins or dioxin-like compounds, Ah-receptor activation results in the increased production of the enzyme luciferase, which can metabolise luciferin, which is measured with a luminometer. In both assay systems, all Ah-receptor active compounds are measured in an integrated way by measuring the total biological response due to dioxins, dioxin-like compounds and other compounds (see section below), expressed as 2,3,7,8-TCCD equivalent concentrations (indicated in this report as bioassay-based bio-TEQs).

Transient Ah inducers

Although these bioassays are relatively specific for dioxins and dioxin-like compounds, samples require an appropriate clean-up procedure for the assays to be reliable as an indicator for these substances. Various compounds other than dioxins, such as PAHs (Machala et al., 2001), nitrogen and sulphur-containing polyaromatic compounds (S-PACs, N-PACs). A range of other compounds, including indoles, flavonoids and some drugs also bind to the Ah receptor and are responsive in cell-based *in vitro* bioassays such as the DR-CALUX, but do not share the full range of multiple effects of dioxins and dioxin-like compounds (Behnisch et al., 2001; Hilscherova et al., 2000). Many of these (non dioxin-like) responsive compounds are known as **transient inducers**, and are usually readily metabolised. In cell-based tests with wild-type cell lines using prolonged exposure times (such as the 96h carp hepatocyte EROD induction test applied in the aquatic effluent studies), the influence of transient inducers is usually considerably less than it is in short term tests (e.g., the 24-hour DR-CALUX), due to the effect of biotransformation. A recent study prepared for RIKZ (Aarts and Palmer, 2002) made a comprehensive inventory of currently known responsive Ah-receptor agonists in the DR-CALUX test and other related *in vitro* and *in vivo* bioassays. Approximately 430 individual compounds were classified as proven or suspected Ah-receptor agonists. Approximately 62% of these compounds were dioxins or dioxin-like compounds (30 PCDDs, 101 PCDFs, 111 PCBs, 17 PCBs, 8 brominated flame retardants); 25% were PAHs and N-, or S- PACs.

Clean-up methods

Selective clean-up sample pre-treatment is usually applied to separate the contribution of the transiently inducing compounds and to avoid erroneous attributions to dioxin-like compounds. In this pre-treatment, acid/base labile and chemically less stable components are removed while dioxins and dioxin-like PCBs are retained. Currently used clean-up methods include solid-phase chromatography based on sulfuric acid/silica columns

(Murk et al., 1996, 1998; Houtman et al., 2004; Stronkhorst et al., 2002), and/or NaOH/silvernitrate/silica columns (Leonards and van der Veen, 2001), sometimes in combination with carbon column fractionation. The combination of all three clean-up methods is usually applied for the chemical HRGCMS analysis (Pluym et al., 1992), and is considered the most selective separation for planar polyhalogenated aromatic compounds. Because the full 3-step method is labor- and cost-intensive, the relatively rapid sulphuric acid/silica based method is commonly used for DR-CALUX based screening of food and animal feed. This clean-up method was also used in the OVOC project.

Validation of acidic clean-up

As became clear during the OVOC project, the presence of high concentrations of PAHs, including some known responsive PAHs, in effluents, vent gases and some products could be a confounding factor. Information that is available from related studies (Schramm et al., 2001) indicates that the presence of some PAHs cannot be ruled out after acidic clean-up, depending on column type, elution solvent and the dimensions of the clean-up system. Additional validation studies (Lamoree et al., 2004b) were therefore executed to test the performance of the acidic clean-up procedure applied in the OVOC project, with respect to the quantitative removal of potentially responsive PAHs and related non-dioxin like compounds. Most of the PAHs with higher molecular weight (i.e., those with four or more rings) are efficiently removed, including the PAHs with the highest activity in the DR-CALUX test. However, several responsive PAHs, benzo(b)fluoranthene (removal 85-90%) and fluoranthene (removal approximately 80%) are not quantitatively removed. The contribution of PAHs (after acidic clean-up) is likely to be low in many cases, as demonstrated in the in-plant TIE study of Senhorst et al. (2004b). Additional verification of the concentrations of responsive PAHs and comparison to their relative effect potency in the DR-CALUX test is required, however, in order to assess the exact relative contribution of PAHs in the final response.

In addition to the specific problem with PAHs, the validation of the acidic clean-up step as applied in this study showed that this sample treatment could not be considered a selection tool for persistent substances. Heptachlor, an easily hydrolysable substance with a half-life in water of only one day (Carey, 1998) was quantitatively recovered after acid treatment, while such known persistent substances as aldrin, dieldrin, isodrin and endrin did not pass the clean-up step. The conclusion should therefore be that there is no strict relationship between the substances that pass the clean-up step and persistency. In this study, the acidic clean-up step is used to pinpoint dioxin-like substances (as these substances are known to pass the clean-up step), while at the same time recognising that signals after acid treatment may also have been induced by transient substances.

For the interpretation of the outcome of the DR-CALUX and Carp-hep EROD tests, the current study makes a clear distinction between results without and including the acidic clean up. Results based on tests with acidic clean-up may be interpreted as indicating the presence of dioxins and dioxin-like compounds, but the contribution of unknown acid-stable non-dioxin-like transient inducers cannot be fully excluded. This is especially true for the wastewater and product studies.

For the most responsive samples, additional HRGCMS analysis was performed to verify the contribution of PCDD/Fs. Further dedicated analysis of contributions from other dioxin-like compounds (PCBs, PCNs) was beyond the scope of the project.

3.5 Identification of substances

Three techniques were used for the identification of substances: GC-MS screening, chlorine specific GC techniques and dedicated HRGCMS analysis for the detection of dioxins (PCDD/Fs).

Gas Chromatography with Mass Spectrometric Detection (GC-MS) is one of the most general analytical techniques available for identification studies. It is able to detect PBTs and other compounds with a wide range of characteristics and molecular masses. This analytical advantage of high selectivity, however, has a negative effect on the sensitivity when applied in screening studies. For GC-MS screening analysis, the sample clean-up is limited or excluded, as this could destroy possibly relevant substances. In this way, however, a whole range of non-relevant substances is accepted into the analysis as well. This is manifest in a relatively high level of background noise, leading to relatively high detection limits (of the order of 0.1 µg/l in effluent samples). This drawback becomes very limiting in the detection of complex mixtures of substances involving large numbers of isomers or homologues. As a result, low concentrations of toxic chlorinated PAHs, PCBs or similar substances can be overlooked due to interfering non-toxic compounds present at high concentrations.

To a certain extent, chlorine-specific GC techniques provide a solution to this problem. The use of such detection methods as NCI-MS or ECD that are susceptible only to electro-negative compounds (e.g., those involving chlorine) can substantially reduce background noise. Atomic Emission Detection (AED), as applied in the in-plant TIE study (Senhorst et al., 2004b) is a technique that can even detect specific isolated chlorine compounds. These chlorine-specific GC techniques have been used in this study for cases in which GC-MS screening did not lead to satisfactory identification.

Dedicated dioxin (PCDD/F) analysis by high resolution GC in combination with high resolution MS (HRGCMS) was applied in a selected set of samples in order to link the results of the DR-CALUX assay to dioxins. The analysis involves extensive sample clean-up, thereby destroying less stable and non-relevant (i.e., non-dioxin) substances. Detection limits for individual dioxin or furan isomers are of the order of 1-10 pg/L. Depending on the TEFs of these isomers, the TEQ-based detection limits can be several orders of magnitude lower.

In addition to detection limits and background noise, another analytical limitation hampers the identification of substances. Formally, identification can be achieved only when there is sufficient matching of a GC-MS peak and mass spectrum with 1) either the characteristics of the identifier substance tested previously under the same laboratory conditions or 2) with the mass spectrum and/or retention index, as published in open GCMS databases. The first approach requires lab standards of the substance under consideration, which is unpractical when screening for unanticipated compounds. It would require lab standards of hundreds or even thousands of reference substances. The second approach can be successful, but only to the extent that reference mass spectra of substances are available in such public databases. This has proven to be a very limiting factor. Section 4.6 deals with these problems in more detail. Identification by means of hyphenated GC techniques is further limited by the fact that not all substances are separable by gas chromatography.

Substances that have a high boiling point, which tend to have high molecular masses, are particularly likely not to be detected. Detection of new, as yet unidentified substances can occur only through the extensive analysis of the mass spectra. This is a very time-consuming affair and is only opportune when e.g., toxicity has been narrowed to a limited number of unknown GC peaks.

4. Description of experimental methods

4.1 Framework

The combined chemical and biological screening of the wastewater samples (Lamoree et al., 2003), was based on a stepwise approach, which started with a chemical screening to confirm the presence of chlorinated substances in pentane extracts, followed by toxicity screening with a battery of *in vivo* and *in vitro* bioassays. The screening subsequently involved fractionation with RP-HPLC and further testing of fractions of responsive samples differing in hydrophobicity, in order to classify the bioaccumulative potency of the responsive compounds, and the execution of biodegradation tests. Consecutive chemical screening with GC-MS-based methods was used to identify individual compounds in responsive samples and fractions, and to explore possible causal relationships. For the atmospheric emissions and products (Van Hattum et al., 2004a,b) a less comprehensive approach was followed, due to budgetary constraints.

The following sections provide a brief description of the methods that were applied. For detailed descriptions of the methods, we refer to the technical reports of the different subprojects, which are included on the Annex CD-ROM.

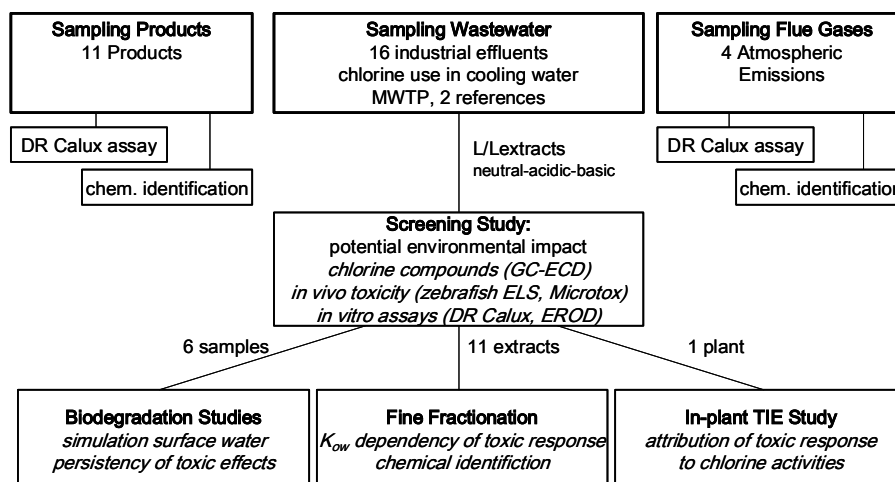


Figure 4.1 Framework of effect-directed biological and chemical screening methods applied in the OVOC project.

After the initial wastewater screening study, a selection of effluents, samples or fractions was studied in the biodegradation (Senhorst et al., 2004a), fine fractionation (Lamoree et al., 2004a) and in-plant TIE subprojects (Senhorst et al., 2004b). The selection (see Lamoree et al., 2003) was based on the amount of DR-CALUX response or ECD signal (biodegradation study), the DR-CALUX response in the coarse fractions (fine fractionation study) and amount of DR-CALUX load that could not unambiguously be attributed to chlorine-chain activities only (in-plant TIE study).

The value of this experimental set-up is that it uses such novel techniques as *in vitro* bioassays in combination with the more validated test methods represented by the *in vivo* toxicity tests. For example, although *in vitro* bioassays have not been used on aquatic industrial effluents before, *in vivo* tests have. Combining both types of information allows the exploration of a new dimension of effluents while still keeping safe ground. In addition, the tiered approach in the experimental set-up makes it possible to focus efforts most effectively while obtaining new information.

4.2 Sampling programme

Samples of effluents and atmospheric emissions were taken under normal operating conditions. Prior to sampling, plant managers were contacted in order to plan the sampling dates for times in which no maintenance programs or other major upheavals were known to occur. Products were obtained directly from manufacturers. For each emission and product, only one sample was used. For this reason, no information is available on representativeness of the substances and effects found for a longer period of time. Such quantified parameters as concentrations or effect loads that are reported in Chapter 5 should therefore be considered only as an *indication* of what might actually be emitted.

Sampling of effluents

Sampling of effluents and reference samples took place in February and March of 2001. At the moment of sampling, company staff signed a statement that all conditions were normal. Of the 16 prioritised industrial effluents, 14 could be sampled: one company (Givaudan Roure) had ceased production of ethylene dichloride; the other company (Atofina, Vlissingen: production of organotin compounds) initially refused co-operation but was sampled later on in 2002. At that time, however, processing of the sample could no longer be combined with other analyses in the study, and the effluent was not included in the study.

Grab samples were taken of the effluent as discharged into surface water, after wastewater treatment, if applicable. One industrial effluent (MDE) was sampled after treatment in a municipal wastewater treatment plant. In order to get information about substances and effects produced by chlorination of the cooling water, the surplus water of a closed cooling-water system treated with hypochlorite was sampled (both influent and effluent).

Three reference water samples were taken: the effluent of a purely domestically loaded municipal wastewater treatment plant (MWTP), surface water of Lake Marken (which is known to be relatively low in organic pollutants) and a sample of (Lelystad) tap water.

Each water sample measured a volume of 70 L, of which 20 L was processed directly, the remainder being stored in cold storage (-18 °C) for later use (biodegradation test, possible re-analysis). Samples were liquid-liquid extracted in pentane (acid, base, neutral) and transferred into methanol, thus yielding extracts with a concentration factor of 1:1000. Validation of this extraction step (Lamoree et al. 2003) showed that substances with $\text{Log } K_{ow} > 2$ are quantitatively recovered. Volatile substances with a Henry constant value above 2 are poorly recovered.

Sampling for the in-plant TIE study (Senhorst et al., 2004b) at site MCD took place in March 2002, after intensive contact with the company in order to select the proper waste water streams and sampling points.

Sampling of atmospheric emissions

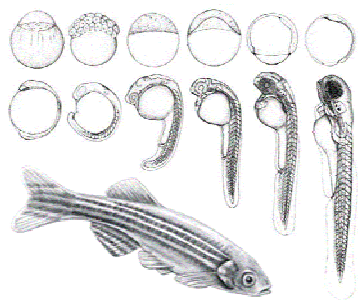
Four atmospheric emissions were sampled: one of a solid waste incinerator and three emissions of industrial vent-gas incinerators (Van Hattum et al., 2004a). The sampling was executed by TNO-MEP-PA (Apeldoorn) according to NEN-EN 1948-1 (NEN, 1997a) with a Ströhlein dilution device, commonly applied for the sampling of PCDD/Fs. A three-part system consisting of a glass fibre filter with additional polyurethane foam (PUF) and active carbon filters was used to trap dust and gaseous compounds. The filter was Soxhlet-extracted with a 3:1 hexane/acetone mixture.

Sampling of products

With the help of the VNCI, samples of eleven products, which were considered representative for the Dutch market, were obtained directly from their suppliers. Seven of these samples were concentrated or extracted in such a way that only the non-volatile impurities and not the original product ended up in the extract, yielding a concentration factor for these impurities of between 1:1000 and 1: 2000. For four non-volatile products, this procedure could not be followed. Three products (triclosan, paradichloro benzene and chloroparaffin) were dissolved in hexane. For these products no concentration of impurities is achieved, making analysis much more difficult. One product (chloroprene rubber) was Soxhlet-extracted and then transferred into hexane (Van Hattum et al., 2004b).

4.3 Biological methods

Short descriptions are provided of the two *in vivo* tests used (8-day zebra fish ELS test, 22h-chronic microtox test), for screening on general semi-chronic toxicity, and the two *in vitro* assays: (DR-CALUX assay, and Carp-hep assay) for screening on dioxin-like toxicity and (anti)estrogenic potency. For detailed descriptions, see Lamoree et al. (2003).



Zebra fish 8-day Early Life Stage test

The ELS test used in this study determines the effect on the egg and larvae phase of the zebra fish (*Brachydanio rerio*). The rate of mortality and the occurrence of malformations are measured in the 8-day period after fertilisation. The protocol of the test is based on the OECD-212 guideline. The test involves 4 replicates, each starting with 25 eggs. For budgetary reasons, the

test was performed as a screening test only at the concentration that was found in the original effluent. The tests were performed by TNO Delft, Department of Environmental Toxicology. A reference test (positive control) involving 3,4-dichloroaniline was applied to check for the validity of the test. From literature data (Elonen et al., 1998, Berends et al., 1991), it is known that the ELS test is more sensitive for dioxins, compared to standard acute tests with daphnids or algae, for example, but less sensitive in comparison to salmonid fish species, such as lake trout (Walker et al., 1991).

22h- Chronic Microtox™ tests



The chronic micro-toxicity test used involves the species *Vibrio Fischeri*, a marine bacterium that produces light. In the course of the test (22 hours), the bacterial concentration increases by a factor of three. Parameters that influence the growth of the bacteria may lead to detectably lower light production at the end of the test. In addition to this source of toxicity, the test is also sensitive to parameters that influence the activity level of the bacteria, to which light production is closely connected. This is the type of toxicity that is tested in the acute toxicity test with *V. Fischeri*. Chronic toxicity tests with *V. Fischeri* are generally more sensitive than are acute tests. Comparative studies have shown that tests with bacteria are comparable to, and sometime more sensitive than, tests with water flea, algae, plants or fish (Gellert et al., 1997, Sweet, 1997, Sloof & Canton, 1993). All tests reported below were conducted with bacterial populations, media and test analysers as supplied by Azur Environmental. All tests were performed by Aquasense, Amsterdam.

Carp Hepatocyte Assay

Carp (*Cyprinus cyprinus*)



The CARP-HEP assay is based on freshly isolated carp liver cells obtained from genetically uniform male carp (*Cyprinus carpio*). In the EROD tests, the induction of P4501A (CYP1A) enzymes is measured in hepatocytes (liver cells), as a measure of the potential of environmental extracts to cause dioxin-like toxicities.

The induction of CYP1A enzymes is a consequence of an agonistic effect on the aryl hydrocarbon (Ah) receptor. Ethoxyresorufin-O-deethylase (EROD) activity is used as a measure of the catalytic activity of CYP1A and thereby the exposure to substances inducing the dioxin-like toxicity. Estrogenic and anti-estrogenic potency were determined based on the induction of Vitellogenin. Additional tests for cytotoxicity were the LDH (lactate dehydrogenase) leakage and MTT (mitochondrial activity) test.

DR-CALUX™ assay



The DR-CALUX® assay¹³ is based on the activation of a reporter gene (fire fly luciferase) that has been introduced in the genetically modified cell-line (rat-hepatoma H4IIE). The stable cell-line expresses fire-fly luciferase genes (in addition to genes that are normally expressed in the parent cells upon exposure to dioxins or dioxin-like compounds). As a consequence, these cells emit light in the presence of luciferin, which is easily quantifiable with a luminometer. The amount of light production is related to the concentration and composition of dioxins, dioxin-like and other responsive compounds in the exposure mixture (Sanderson et al., 1996, Murk et al., 1996). A full

¹³ Dioxin Responsive Chemical Activated Luciferase gene expression assay.

list of references on mechanisms, performance characteristics and applications are provided at www.biodetectionsystems.com). All Ah-receptor active compounds are measured in an integrated way by expressing the total biological response as 2,3,7,8-TCCD equivalent concentrations (known in this report as bio-TEQs).

4.4 Status of *in vitro* methods applied in study

A brief summary of the performance characteristics, validity and acceptance of the Carp-Hep and DR-CALUX assays is presented in the Appendix section (9.4). The DR-CALUX method has been validated extensively as a screening tool for dioxins and dioxin-like PCBs in various matrices (especially food, feed, sediments), and meets the criteria defined by the EC-food directive of 2001 for performance characteristics. It has been applied successfully as a screening tool for the presence of dioxins and dioxin-like PCBs in accidental food poisoning incidents and in common regulatory screening of food-products and animal-feed materials (Overmeiere et al., 2001; Schoeters et al., 2004). In the Netherlands, the DR-CALUX has further been validated for the screening of harbour dredgings (Stronkhorst et al., 2002), and is included in the regulatory framework for the evaluation of offshore disposal of harbour dredgings, expected to take effect in 2004. Neither of the two *in vitro* methods had been validated for application in effluent screening studies prior to the OVOC project.

False negative: False negatives (i.e., lack of response in the test when responsive compounds are present) may occur when: (1) sample extracts are cytotoxic, which is easily identified by examination of the condition of the cells using specific tests (LDH, lactate dehydrogenase leakage; MTT, a parameter for mitochondrial activity; protein loss); (2) samples inhibit luciferase or EROD activity (rarely completely blocks response, thus some induction will usually be observed; and (3) active components are lost during extraction or clean-up. The frequency of false negatives in the DR-CALUX test is very low, as demonstrated in validation studies for the screening of food products (Schoeters et al., 2004) and harbour dredgings (Stronkhorst et al., 2002).

False positives: Although the *in vitro* methods applied in the OVOC study are highly responsive and specific for dioxins and dioxin-like compounds, several other compounds (transient inducers, see Section 3.3) may also induce a response, although they do not share the full range of multiple effects of dioxins and dioxin-like compounds. Because many of these transient inducers are not chlorinated, this may give rise to a false-positive response within the framework of the OVOC project.

Both tests can be used for prescreening (selection for GC-MS analysis or further fractionation, or for other toxicological investigations), ranking (provides a rapid and cheap first impression of sample activity) and estimation of bioassay-based TEQs (after appropriate clean-up to eliminate potential false positives (see Section 3.3). The DR-Calux, based on a genetically modified mammalian liver cell (rat-hepatoma), provides limited information about species sensitivity to dioxin-like chemicals. The Carp-Hep assay provides some idea of the relative sensitivity of the Carp compared to other species but provides only little relevant information for ecological risk assessment.

In summary, both tests are sufficiently reliable and sensitive for the screening purposes of the OVOC project and, when the appropriate cleanup methods are applied, both tests

can be used as indicators for the presence of dioxins and dioxin-like compounds. The contribution to the estimation of ecological risks is limited. For this purpose, these tests should always be used in combination with information obtained from *in vivo* bioassays and from field studies.

For the interpretation of the outcome of the DR-CALUX and Carp-hep EROD test, the current study makes a clear distinction between results without and including the acidic clean-up. Positive results found in tests with extended fractionation and acidic clean-up, may be interpreted as an indication for dioxins and dioxin-like compounds. The outcome of the validation studies, however, indicates that the contribution of unknown acid-stable non-dioxin-like compounds cannot be excluded. Especially for wastewaters of complex composition, this issue must be addressed separately.

4.5 Fractionation of extracts

Lamoree et al. (2003, 2004a) provide a detailed description of the reversed-phase HPLC fractionation procedures applied in the OVOC study and the validation of these procedures. A brief description appears below.

In order to determine the Log K_{ow} of substances and mixture, the extracts produced in the sampling campaign were fractionated into separate Log K_{ow} classes. This was accomplished in two steps. First, a rather coarse type of fractionation was applied involving reversed-phase High Performance Liquid Chromatography (HPLC) with methanol as eluent. This yielded three fractions with Log K_{ow} intervals of approximately below 4, between 4 and 6 and above 6, respectively. The cut-off values for the retention time of these Log K_{ow} boundary values were determined by fractionation of the EPA mixture of 16 PAHs.

In order to narrow down the Log K_{ow} range to a smaller interval and to create more sharply defined intervals, a second, more refined fractionation step was applied, which involved reversed-phase HPLC using a methanol/water gradient. A mixture of chlorinated pesticides and PCBs was used to calibrate the Log K_{ow} boundary values. The fine fractionation step yields extracts with Log K_{ow} intervals as small as $\Delta \text{Log } K_{ow} = 1$.

4.6 GC-MS identification studies

In several work packages of the OVOC study, GC-MS analysis is used as a screening tool for identifying the presence of substances in extracts of wastewater, air emissions or products. As indicated in Figure 3.1 (scheme of target compounds) the presence of chlorinated substances is heavily emphasised in identification. In addition to this use as screening tool, dedicated HRGCMS analysis was used in the OVOC project for the detection of PCDD/Fs. A detailed description of analytical methods involved is provided in the reports of the various OVOC subprojects.

The OVOC study made use of two types of identification (see Appendix 9.6):

- Tentative identification: based on automated matching of recorded mass spectra with NIST mass spectral data base (contains over 100,000 substances), combined with filtering on chlorine containing substances and a manual check on match of mass spectra. This yields a *likely* identification of substances involved, although there still

is a chance of erroneous attribution due to (stereo)-isomers or closely related compounds. Note that the tentative identifications presented here are not yet conclusive. For a complete identification, the mass spectrum and the retention index of the component in the sample should be compared with pure standards measured under the same conditions on the same instrument (Bobeldijk-Pastorova, 1998; EPA, 1989). As such procedures are clearly beyond the scope of the OVOC project, we follow the EPA definition for 'tentative identification' when a matching with mass spectra from an external library can be demonstrated. This approach was also used in the GC-MS screening studies for surface waters (Barreveld, 2001; Staeb et al., 2002) in the Netherlands.

- Indicative characterization: no sufficient matching with existing reference mass spectra in the NIST database. Despite the vast amount of mass spectra recorded in the NIST database, this resource tends to give only matches with smaller, less complex and not highly chlorinated compounds. A manual search strategy was adopted to find chlorinated elements in mass spectra in order to gain more complete insight into the presence of other chlorinated compounds. Although this strategy cannot identify the corresponding compound directly, it yields information on the degree of chlorination (number of chlorine atoms in a molecule) and also provides a lower estimate on the molecular mass. This indicative characterization was used in the analysis of product extracts in order to get a more complete picture on the presence of chlorinated impurities. It does not identify substances in the strict sense of the word, but rather leads to the assignment of chlorinated substances. For the sake of brevity, this report uses the term *indicative characterisation*.

As an example, Figure 4.2 provides the results of both types of identification in the analysis of the extract of trichloroethylene. It shows that the tentatively identified compounds fall in the retention time (RT) window up to 7.27 minutes, while the group of indicatively identified compounds extends to RT = 10.36 minutes. Because of the GC method used, retention time roughly scales with molecular mass (Figure 4.3).

These results indicate that identification by means of the NIST database gives a negative bias towards substances with high molecular weight. As within chemical families, the Log K_{ow} usually increases with increasing molecular weight this bias may also hold for higher Log K_{ow} substances.

4.7 Persistency-testing of aquatic effluents

In order to assess the persistency of the toxicity observed in the screening studies, a biodegradation test simulating the residence of effluents in the water column in surface water was applied (Senhorst et al., 2004a). Because of the salinity of most effluents in the OVOC study, and because of the proximity of most of the discharges to coastal waters, all effluents were degraded in salt water.

This facilitates the comparability of the results and provides an indication of the relative potential for biodegradation within the group of tested effluents. The results of the biodegradation test, however, cannot be interpreted as a description of the fate of each effluent in its specific receiving surface water. Such descriptions require dedicated degradation tests simulating the specific conditions of each effluent and its receiving surface water.

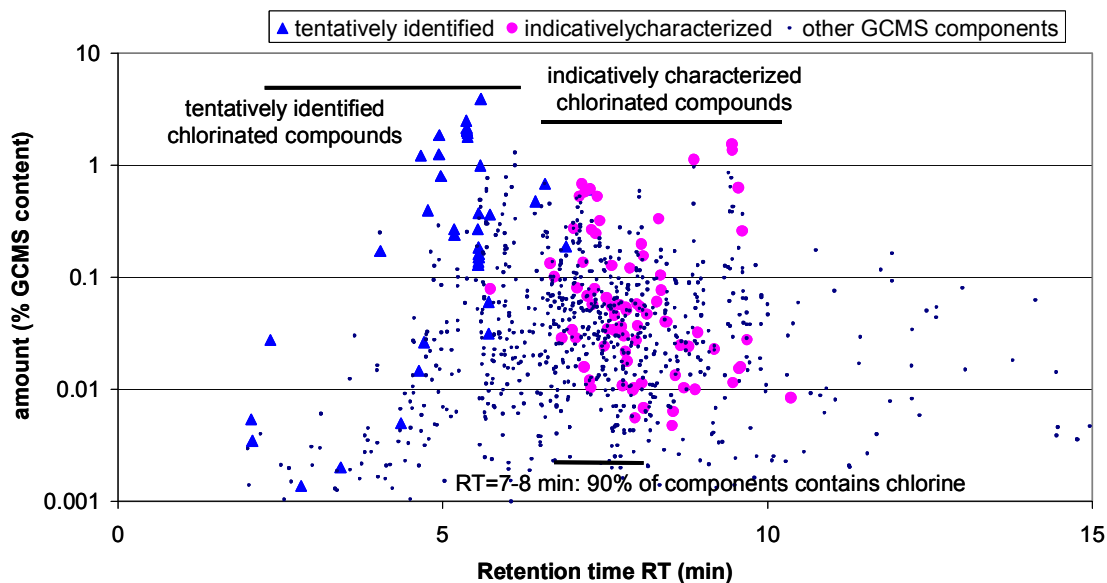


Figure 4.2 Comparison of results of tentative identification (triangles) and indicative characterization (circles) of chlorinated compounds for the analysis of trichloroethylene. Dots: other non-chlorinated or unidentified components.

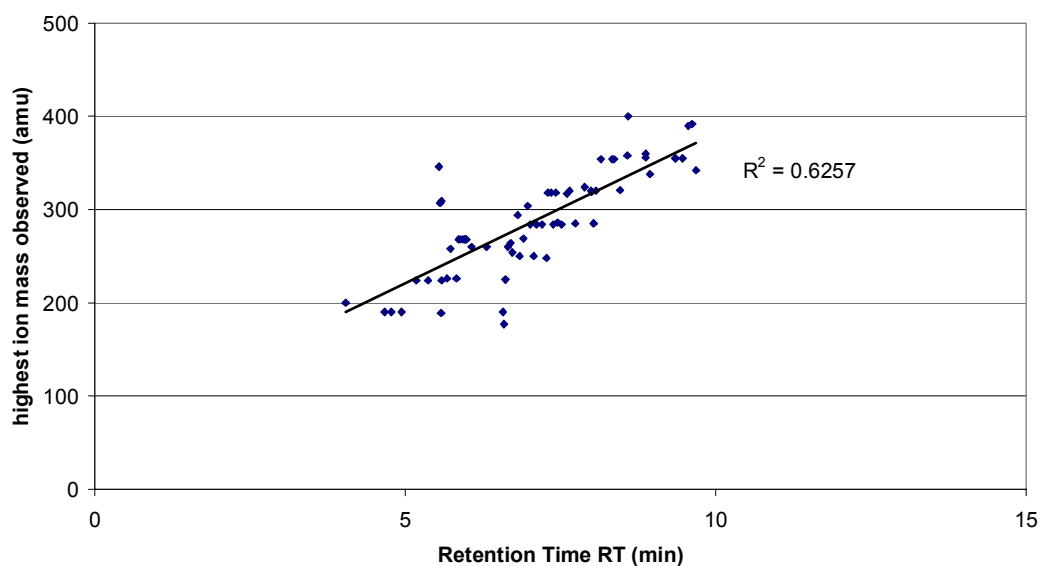


Figure 4.3 Relation between the highest molecular mass (m/z) observed in the mass spectra of chlorinated components in the sample of trichloroethylene as a function of retention time. The line drawn is the linear regression through the data points.

The test protocol was developed by Aquasense, under authority of RIZA (Aquasense, 2001). It is based on the OECD guidelines 301E ('Modified OECD screening test') and 306 ('Biodegradability in seawater') and simulates (bacterial) biodegradation in the aquatic environment, in either fresh water or salt water. The test allows for an evaluation of biodegradation effects over a 28-day time period in marine water. The present guidelines in international policy frameworks (see Appendix 9.5) for establishing persistency involve a period of between 40 to 60 days. Explicit interpretation of the test in this context requires a correction based on an extrapolation assuming first-order decay. Within the OVOC study, however, a more qualitative approach was assumed to suffice, and it is unlikely that the shorter exposure period would have led to fundamentally different conclusions.

The test protocol involves 1:1 mixing of effluent with reference surface water (taken from the Eastern Scheldt). The bacteria and other micro-organisms present in this reference water act as inoculum for the biodegradation. The solutions are aerated and left in the dark at a temperature of 15°C. A positive control involving anilin is used to check for the occurrence of biodegradation. After the biodegradation step, the solutions are liquid/liquid extracted by the same procedure as applied to the 'fresh' effluent samples in the screening phase.

5. Results and discussion

This chapter provides a selection of the OVOC results that are considered most relevant in light of the two main research questions of the project, as described in Chapter 1. Not all results could be included. We refer the reader to the reports of the separate subprojects for a review of the detailed results and more methodological issues. All technical reports are included on the Technical Appendix CD-ROM.

5.1 Chlorinated substances identified in emissions and products

Effluents

AOX and EOX: In the screening phase, the effluents were characterised by their content of adsorbable and extractable chlorine content, AOX and EOX respectively. Levels of these parameters reached up to the order of mg/l. Results indicated that a large part of this chlorine content consists of volatile substances. Furthermore, the distribution of the ECD signals (electronegative compounds, which included chlorine-containing compounds) over the three Log K_{ow} fractions peaked at the low Log K_{ow} fraction, leaving only sub $\mu\text{g/l}$ signals in the high Log K_{ow} fraction. This shows the dominance of more polar compounds in the effluents.

Individually identified substances: The observation above, that there is a tendency for chlorinated substances in effluents to be polar in nature, is further corroborated by the results of the in-plant TIE study performed at site MCD (Senhorst et al., 2004b). These results revealed an abundance of chloro(di)propyl ethers in the low Log K_{ow} fraction (Log $K_{ow} < \pm 4$) of both untreated wastewater of the chlorine activities and effluent from the site. These substances made up a considerable part of the EOX measured in the samples. Less abundantly, a number of unknown chlorine-containing compounds were found in the medium Log K_{ow} fraction (Log K_{ow} between 4 and 6) of the chlorine activities in untreated wastewater (but not in the effluent). The high Log K_{ow} fraction revealed no chlorinated compounds, neither in the chlorine activities untreated wastewater nor in the effluent.

In the responsive effluents studied, no organochlorine compounds could be detected in the fine high Log K_{ow} fractions, either by GC-MS or GC-NCI-MS screening procedures (Lamoree et al., 2004a).

For the effluents, dioxin analyses by HRGCMS was performed only on the medium and high Log K_{ow} fractions of the MCD effluent sampled in the in-plant TIE study. Not all DR CALUX responsive samples could be analysed because of budgetary constraints. Table 5.2 presents these results, together with the results of PCDD/F analyses of the atmospheric emissions and three products.

Atmospheric emissions

GC-NCI-MS screening of the high Log K_{ow} fractions of the atmospheric emissions revealed penta- and hexa-chlorobenzene, as well as an unknown tetra-chlorinated compound. In addition, a compound with a monochlorophenol fragment was detected.

The concentrations of these substances varied between approximately 0.1 and 80 $\mu\text{g}/\text{Nm}^3$. Table 5.3 provides more details.

Products

The results of only eight of the 11 products are reported here. For the other three products (triclosan, 1,4-dichlorobenzene, and C_{14-17} -chloroalkanes), the standard screening approach with GC-MS for concentrated residues was not successful in identifying chlorinated components at levels below 1%, due to problems related to chromatographic separation, selectivity or sensitivity, as well as to the fact that no appropriate concentration could be achieved due to limitations in sample preparation. In the eight successfully analysed products, a range of chlorinated impurities was found using both the tentative identification method and the indicative characterisation method (See Section 4.6).

Tentative identification: A total of 45 chlorinated chemical substances were tentatively identified in 8 of the 11 tested products. Both the amount and the nature of the identified chlorinated compounds varied strongly from product to product. Section 5.5 provides a full list of these identifications and discusses them in more detail.

Indicative characterisation: The main results of the indicative characterisation method used are that, in each GCMS chromatogram, many more components are attributable to chlorinated compounds: the chlorine content is typically multiplied by a factor between 0.5 and 10 (and, in one case, >100), relative to the result of the tentative identification. Table 5.1 presents an overview of the identification results.

Table 5.1 Results of the analysis of the product samples by tentative identification as well as indicative characterisation¹.

Product	Content tentatively identified chlorinated compounds (mg/kg)	Content indicatively characterised chlorinated compounds (mg/kg)	Degree of chlorination	Character of chlorinated compounds
chloroprene rubber	10-100	10-100	1,2,3	benzene, cyclohexene, butadiene, cyclo-octadiene
trichloroethylene	0.2-2	0.1-1	1,2,3,4,5,6, (8)	butadienes, butenes, hexadienes, benzenes, naphthalene
allylchloride	0.1-1	1-10	1,3,4,5,6	propene, propane, pentene, hexane, hexadiene
monochloro acetic acid	0.01-0.1	0.1-1	2,3,4	alkylated benzene, xylene
1,2-dichloroethane	0.002-0.02	0.01-0.1	1,2,3	esters, ethers, benzene groups
perchloroethylene	0.002-0.02	0.001-0.01	1,3,5,6	alkanes, alkenes
hydrochloric acid	0.001-0.01	0.01-0.1	2,3,4,5,7	Benzene, phenol, xylene
dichloromethane	<0.00003	<0.005	1,2,3	Propene, benzene

¹ For explanation of status of the identifications, see Section 4.5.

Source: OVOC-report Van Hattum et al. (2004b).

For four products, the type of chlorinated compounds identified or characterised definitely shows a specific regularity that is not present in the results of other products. A link with the production process seems obvious in several cases.

Allylchloride: Although not identified by the NIST mass spectral database, the GC-MS shows the presence of a range of related compounds that, based on literature data (De Leer, 1986), can be attributed to chloropropyl (mono/di/tri) ethers. These ethers are formed in the production of epichlorohydrin (for which allylchloride is a precursor) and were also present in the influent and effluent of production site MCD where allylchloride is processed¹⁴. Chloropropyl ethers are well known by-products of epichlorohydrin production. They are poorly degradable under aerobic conditions, are moderately toxic, possibly mutagenic and have Log K_{ow} values between 3 and 4 (RIZA, 1995).

Monochloroacetic acid (MCA): Indicative characterisation yields several chlorinated methyl/ethyl benzenes. When searching for corresponding mass spectra of other isomers or family members, a range of chlorinated alkyl benzenes is found: fully substituted benzenes with either a chlorine atom or a methyl or ethyl group as substituent, with the amount of chlorine atoms being 2, 3 or 4. For MCA, an intensive analysis program has been started by the company involved to determine the origin of the components found. The raw materials used to produce MCA are not based on aromatic feed stocks, and no aromatic compounds are used or added in the process. If possible, the program should lead to elimination of the sources.

Trichloro-ethylene: Tentative identification yields substantial amounts of (isomers of) pentachlorobutadiene. This substance can be formed from two trichloroethylene molecules under the removal of HCl (hydrochloric acid). This reaction is the first step in the condensation polymerisation with trichloroethylene as a precursor. Further reaction steps would produce such linear substances as chlorinated hexatrienes and octatetraenes or, with ring closure, highly chlorinated benzenes and benzenevinylidenes. The indicative characterisation method indicates the presence of these substances.

Chloroprene rubber: Tentative identification yields chlorinated benzene, cyclohexene and cyclo-octadiene as impurities. These substances are either breakdown products of polychloroprene (e.g., produced in the Soxhlet extraction) or are non-reacted precursors of polychloroprene still present in the product. Poly-chloroprene is produced from the 2-chloro-1,3-butadiene monomer.

Non-chlorinated compounds observed

In addition to the chlorinated compounds, the GC-MS analysis (tentative identifications) revealed the presence of a number of non-chlorinated substances in significant amounts in a number of product extracts. At issue are widely used plasticisers and anti-oxidants that are added to the products. Analysis of the chloroprene rubber sample showed the presence of several 4-ring PAHs and PAHs with higher molecular weight at a total level of approximately 50 mg/kg. Among these PAHs a number of known DR-CALUX inducers was found: benzo(k)fluoranthene and indeno(1,2,3,cd)pyrene in extracts without acidic clean-up.

¹⁴ The fact that the by-products of the subsequent process step are detected in allylchloride itself can be explained by recycle loops in the production process.

5.2 Toxicity screening of emissions and products

Effluents

The results of the toxicity screening of the aquatic effluents are discussed below and summarised in Figure 5.1.

In vivo toxicity: One significant result of the *in vivo* studies is that the (OECD validated) zebra fish ELS test does not show any toxicity at all. Because the positive control, with the addition of dichloranilin as a check for proper test results, did show toxicity, the test results are valid. Apparently, the substances in the effluent extracts do not induce toxicity in this test at the concentration as in the original effluent. The chronic Microtox test did show a response in 7 of the 21 samples studied, including the cooling water sample and the municipal waste water treatment plant (MWTP). In the most responsive sample, this response disappeared at a 12-fold dilution with respect to the original effluent concentration.

	Code	in- vivo		in- vitro				Selected for further testing
		Zebrafish ELS 8-d	Microtox 22-h	Carp-hep estrogenicity.	Carp-hep anti-estrogenicity.	Carp-hep EROD	DR-CALUX (no acid cleanup)	
Industrial	MPQ							
	MDE							b
	MIJ							f,
	MAB							f,b
	MBC							
	MJK							
	MKL							
	MOP							
	MEF							f,b
	MFG							
	MGH							
	MCD							f,b,ip
	MLM							
	MRS							
MNO							b	
MQR								
Cooling water	MST							f
Munic. WWTP	MMN							f,b
Lake Marken	BTU							
Tap water	BUV							

Figure 5.1 Classification of *in vivo* and *in vitro* toxicity of extracts of aquatic effluents. Samples with a significant response are indicated in grey.

Note: Samples selected for further testing are indicated in the last column (f: refined fractionation; b: biodegradation; ip: in-plant TIE studies). *In vitro* tests on total extracts without acidic clean-up. Carp-Hep EROD and DR-CALUX results may include contributions from transient inducers (see Section 3.4).

Source: OVOC-report Lamoree *et al.* (2003).

The chronic Microtox test was also applied to six samples after the biodegradation test. In one case, sample MMN, a fourfold reduction of toxicity¹⁵ could be observed, while three other samples still proved to be toxic after the biodegradation step.

In vitro toxicity: As Figure 5.1 indicates, the DR-CALUX assay appears to be the most responsive of the toxicity tests used in this study. In most cases, responses from the other tests tended to coincide with responses in the DR-CALUX assay (only Microtox of sample MRS and the anti-estrogenicity result of sample MNO behaving differently).

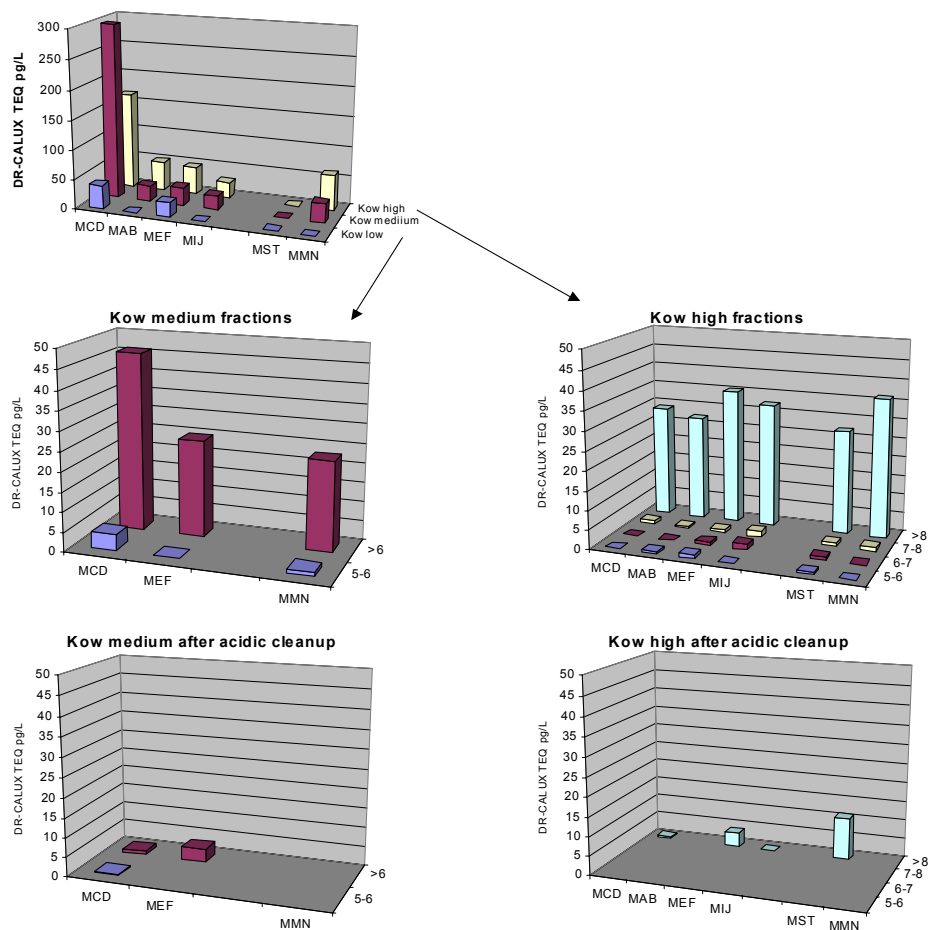


Figure 5.2 Results of detailed RP-HPLC fractionation studies and application of acidic clean-up for the 4 responsive effluents, cooling water (MST) and a municipal WTP (MMN).

Note: On the vertical Z-axis the DR-CALUX-based TEQ concentrations are given in pg bio-TEQ/L. On the horizontal Y-axis the Log K_{ow} intervals of the different fractions are given. For the initial coarse 3-step fractionation, the cut off intervals are indicated as low, medium or high, corresponding with indicative Log K_{ow} intervals of <4, 4-6 and >6. Source: OVOC reports Lamoree et al. (2003, 2004a)

¹⁵ LOEC (expressed as % dilution of effluent) before and after biodegradation: 33% and 133% resp.

Of special interest is the correlation between the carp hep EROD results and the response of the DR-CALUX assay. In nine of the 14 DR-CALUX positive samples, this response coincides with a response in the carp hep EROD test, with the latter test giving no response without a DR-CALUX signal. Because the two tests probe two separate sub-processes in the toxicity mechanism of dioxin, this correlation suggests that the responses are indicative of a true dioxin-like toxicity mechanism (see Section 4.3), although it is important to note that, in tests without acidic clean-up, the influence of transient inducers cannot be excluded. The 96-h EROD test is less sensitive for transient inducers, due to the increased role of biotransformation in comparison to the 24-hour DR-CALUX test.

The distribution of the DR-CALUX response over the three Log K_{ow} classes (Figure 5.2) shows that, in all samples, the major contribution was observed in the medium and high Log K_{ow} fractions. Consecutive finer fractionation of the nine most responsive extracts into $\Delta \text{Log } K_{ow}=1$ intervals showed that the response was always confined to the highest interval studied (Log $K_{ow} > 6$ or > 8). Figure 5.2 presents an overview.

The application of fine fractionation on the coarse fractions can lower the DR-CALUX response, but the amount of this decrease varies from sample to sample. For the high Log K_{ow} fraction of the MST sample no decrease is observed, while for both the medium and high Log K_{ow} fraction of sample MCD a decrease by a factor of 5 is observed. This reduction of response over the fine fractionation may be explained by an increased effect of metabolisation of substances in the (living) test cells of the DR-CALUX assay. Fractionation leads to a smaller spectrum of substances that is offered to the test cells, which can thereby metabolise non-persistent substances more effectively. In the case of sample MCD, the in-plant TIE study showed that a considerable amount of the effluent DR-CALUX response without acidic clean-up originated from PAHs. These substances are known to be metabolised to some extent in the test cells of the DR-CALUX assay.

The decrease of response in the course of fractionation could also be explained by a limited recovery, leading to loss of material. Recovery losses may certainly contribute to the observed decrease, but it is unlikely that this effect is large. First, in the validation of the fine fractionation procedure (Lamoree et al., 2004) low recoveries were reported only for very volatile substances that would disappear in the evaporation step. For compounds like PCBs, drins and PAHs, no problems are mentioned with respect to recovery. Second, the effect of fractionation is quite different for the separate samples: the response of sample MCD decreases by a factor of 12 between total extract and sum of fine fractions (no cleanup) and even by a factor of 20 between coarse fractions and fine fractions (with cleanup), see Appendix 9.9. On the other hand, sample MEF shows a decrease by only some 40% between total extract and sum of fine fractions (Lamoree et al., 2004). This sample-specific decrease cannot be explained by recovery losses alone. Third, the Log K_{ow} distribution appears to shift significantly during fractionation: from responses in both middle and high Log K_{ow} fractions (4-6, and >6 respectively) after coarse fractionation to a response that is confined to the highest Log K_{ow} interval (>6 or >8) after fine fractionation. Recovery losses alone are also incapable of explaining this effect.

Application of the acidic clean-up step considerably lowers the response in all of the fractions studied. For the Log $K_{ow} > 8$ fine fraction of the high Log K_{ow} fraction of MCD,

this decrease is by a factor of 50; for the corresponding fine fraction of sample MST this decrease is by (only) a factor of three.

The DR-CALUX response without acidic cleanup after biodegradation was higher than it was before the biodegradation step (see Section 5.5). This unanticipated result was explained by the occurrence of natural DR-CALUX-inducing substances in the control substrate (Lake Oosterschelde water), or by formation of responsive breakdown products during the further degradation/decay process in the biodegradation step. Application of the acidic clean-up step to the biodegraded extracts lowered the DR-CALUX response to below the detection limit for most of the samples studied. Only sample MCD showed a response after biodegradation and acidic clean-up, but this response was not properly quantifiable.

For effluent MCD, a broad set of results is available from screening phase and fractionation studies (Lamoree et al. 2003, 2004a), biodegradation studies (Senhorst et al., 2004a) and in-plant TIE studies (Senhorst et al., 2004b), both with and without acidic cleanup. Analysis of the set of DR-CALUX responses of these extracts shows that (see Appendix 9.9):

- The DR-CALUX response has a sufficient reproducibility, and responsible compounds were sufficiently stable during frozen storage. The response of the coarse extracts of separately prepared extracts (one year in between) differs by only 15 % ;
- The effect of fine fractionation on the DR-CALUX response (after acidic clean-up) is a decrease by a factor of 20;
- The biodegradation test (Appendix 9.8) does not appear to cause a decrease in the response (after acidic clean-up);
- In the in-plant TIE study (2002), the response in the coarse fractions (after acidic clean-up) is a factor of six larger than in the screening phase (2001), indicating a marked temporal variability in effluent concentrations.

The results of the in-plant TIE study (Appendix 9.7) show that, for site MCD, the response (after acidic clean-up) originates mainly from chlorine chain activities.

The results described above indicate that (fine) fractionation and acidic clean-up independently influence the DR-CALUX response. As metabolism and acidic cleanup both bear a relation to persistency, this conclusion is of significance in interpreting the environmental impact of the DR-CALUX loads (see Section 5.7).

Atmospheric emissions

The DR-CALUX response of the four atmospheric emissions was determined both with and without acidic clean-up. Figure 5.3 presents the result, showing that, for two emissions (270-01- and 270-04), there is a considerable difference between the two types of results. This difference is explained by the occurrence of PAHs in these emissions (van Hattum et al., 2004a). In addition, the results of the PCDD/F analysis are included, demonstrating the part of the response that can be explained by the presence of PCDD/Fs (see Table 5.2).

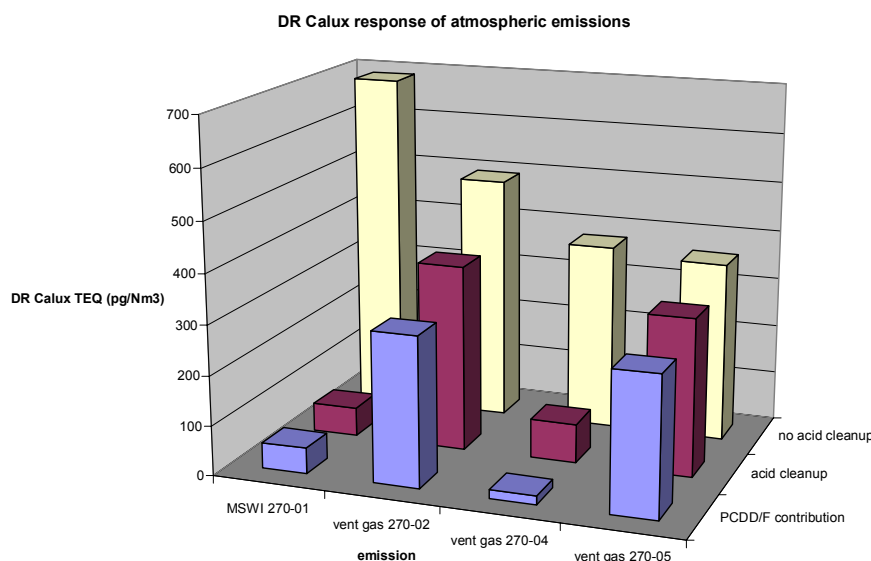


Figure 5.3 DR-CALUX response of atmospheric emissions (pg bio-TEQ/Nm³). MWSI denotes the municipal solid waste incinerator.

Note: The PCDD/F contribution indicated is based on the data from Table 5.2.

Source: OVOC-report Van Hattum et al. (2004a).

Compared to the effect on the aquatic samples, the influence of acidic cleanup is rather limited for two emissions (vent gases 270-02 and 270-05). For these emissions, a large part of the response can be explained by PCDD/Fs. Apparently, the contribution of non-dioxins is very limited in this case.

Products

The DR-CALUX response of all products was determined both with and without acidic clean-up. For dichlorobenzene, triclosan and chloroalkanes, the detection limit was relatively high, due to low test concentrations that had to be used for these products in order to avoid interference of cytotoxicity. Three products showed well quantifiable responses: trichloroethylene, hydrochloric acid and chloroprene. For these products, the contribution of PCDD/Fs is also provided.

5.3 Linking identification and effects

Having presented the results of both the identification and the toxicity studies, we can try to link the two types of results in attempt to identify the toxicity observed. A major obstacle, however, is the extremely small number of chlorinated components that have been identified in responsive effluent fractions and, to a lesser extent, the fractions of the atmospheric emission gas samples.

This lack of proper identifications was unanticipated, and the application of scheduled multivariate statistical analysis could not be executed. Except for the results on PCDD/F concentrations in samples, only qualitative considerations can be provided.

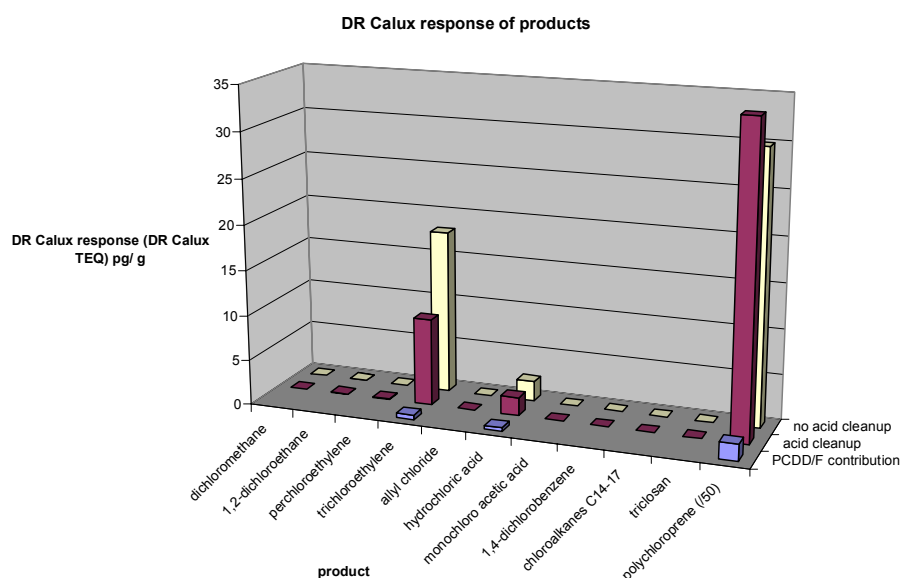


Figure 5.4 DR-CALUX response measured in product extracts (pg-bio TEQ/g).

Note: Measurements below the detection limit are shown by a value in the x-y-plane. The PCDD/F contribution indicated is based on Table 5.2.

Source: OVOC report, Van Hattum et al. (2004b)

Effluents

Except for effluent MCD, no information is available on the presence of chlorinated substances. The observed DR-CALUX toxicity in these effluents therefore cannot be linked to chlorinated substances.

For effluent MCD, three types of substances were found:

- Chloropropyl (di)ethers. These compounds were confined to the low Log K_{ow} fraction in the samples of the in-plant TIE study. They are known to be moderately toxic and could thus be responsible for part of the Microtox observed in the effluent. Because of the varying levels of dependence on Log K_{ow} , chloropropylethers cannot explain the DR-CALUX response.
- PAHs. These compounds were found in the in-plant study samples without acidic clean-up and could explain the major part of the DR-CALUX response and Microtox without clean-up. With clean-up applied, the PAHs in effluent MCD are not likely to contribute significantly to the DR-CALUX response.
- Unknown chlorinated compounds in the medium Log K_{ow} fraction. As these substances do not appear in the high Log K_{ow} fraction, they probably cannot explain the observed DR-CALUX response.

The results of the dioxin analysis can be used to calculate the part of the DR-CALUX response induced by dioxins. The HRGCMS data on concentrations of the relevant PCDD/F congeners and the relative potency of each congener in inducing DR-CALUX response, described by the so-called REP factors (see Appendix 9.4 Table 9.4.2) can be used to calculate a total dioxin-induced DR-CALUX response.

Table 5.2 Measured PCDD/F concentrations in selected samples: comparison with total DR-CALUX response (after acidic clean-up) and response attributable to the contribution of PCDD/Fs.

	Sum PCDD/PCDFs	HRGCMS – TEQs ¹			DR-CALUX based bio-TEQs Incl. acidic clean-up mean ± sd	Explained response
		I-TEQs	WHO-TEQs	DR-Calux REP- based TEQs		
Aquatic effluents			pg/L		pg/L	
MCD medium Log K _{ow} ²			1		170	<1%
MCD high Log K _{ow} ²			0.01		60	<1%
Atmospheric emissions	ng/m ³	pg/m ³	pg/m ³	pg/m ³	pg/m ³	
MSWI 270-01	0.44	36	40	52	58 ± 6	62-90%
Vent gas 270-02	2.23	209	236	300	373 ± 12	56-80%
Vent gas 270-04	0.27	12	13	17	79 ± 5	15-22%
Vent gas 270-05	29.35	137	121	278	316 ± 13	38-88%
Products	pg/g		pg/g	pg/g	pg/g	
Chloroprene rubber	1263		90	93	1700 ± 83	5%
Trichloroethylene	7.8		0.7	0.5	10 ± 0.3	6-7%
HCl	1.7		0.3	0.4	2 ± 0.1	17-21%

¹ Congeners below LOD not included; ² sample taken in in-plant TIE study.

Source: OVOC-reports Senhorst et al. (2004b), Van Hattum et al. (2004a, 2004b).

Table 5.2 presents the result for the dioxin analyses of both effluent samples, atmospheric emissions and products. For the purpose of comparison, the table also presents the corresponding I-TEQ and WHO-TEQ values.

For the in-plant TIE sample of effluent MCD, less than one percent (< 1%) of the DR-CALUX response (after acidic clean-up) can be explained by the presence of dioxins. This result coincides with the results of the extensive analysis of MCD samples (Appendix 9.9) which show that the DR-CALUX response (after acidic clean-up) can be reduced by fine fractionation, probably due to an enhanced metabolism in the more diluted fine fractions, by a factor of 20 (see also Section 5.2.1).

Atmospheric emissions

Both the *in vitro* screening and the chemical analysis of dioxins demonstrated the presence of dioxins and dioxin-like compounds in flue gases from all four installations. Biologically determined TEQ levels were higher (60 to 370 pg bio-TEQs/Nm³) than were measured PCDD/F concentrations (12-209 pg I-TEQ/Nm³; using I-TEFs). In three of the four samples, as much as 80 to 90% of the DR-CALUX response could be explained by the presence of PCDD/Fs, using the DR-CALUX relative potencies; in one vent-gas installation only as much as 22% could be attributed to dioxins. The unexplained part of the response in this sample is attributable to unknown compounds, which could be classified as potentially bioaccumulative acid-stable compounds. In a number

of atmospheric emissions, penta- and hexa-chlorobenzene were identified (see Table 5.3), but these compounds are not known to be responsive in the DR-CALUX test.

Products

The types of chlorinated substances identified in the product samples are listed in Tables 5.1 and 5.7. Of all the compounds mentioned, none is known to induce DR-CALUX response¹⁶. For this reason, only the contribution of dioxins, as shown in Table 5.2, is left to explain only a minor part of the DR-CALUX response. In principle, PAHs might be relevant for the response in chloroprene rubber as smaller PAHs are known to survive the acidic clean-up. However, based on the expected removal of responsive PAHs during acidic clean-up (Lamoree et al., 2004b) and the observation that the responses before and after acidic clean-up do not deviate significantly, the probability of contributions from PAHs to the DR-CALUX response (both with and without acidic clean-up) in chloroprene rubber is expected to be low.

5.4 Loads of substances and toxic-equivalents

From the measured concentrations of substances, the responses of the DR-CALUX assay and the volumes of emissions and products annual loads can be calculated and used in the environmental assessment of the emissions. However straightforward this calculation may be, a word of caution is in order. As the data of only one sample of each emission or product is used in the calculation, the result is vulnerable for the fluctuations that each process is likely to have. The effect of the random variability of individual effluents decreases when cumulative loads of a number of emissions are considered, but care should be taken in interpreting the emission load values nonetheless. Load values presented in this report should be considered only as an *indication* of actual loads.

Emission loads via effluents

Effluent volumes as given by companies were used in the calculation. A different procedure was used for the cooling water sample (MST). The cooling water sample was considered representative of all cooling water discharges in the Netherlands that use hypochlorite as biocide. The equivalent flow volume of all these cooling water discharges was therefore used in the calculation. For more details, see Lamoree et al. (2003).

Chlorinated substances: For the parameters AOX and EOX, the annual loads emitted by individual effluents vary from less than a few kg/y to as much as 45 tonnes/y. The loads corresponding to the GC-ECD response in the extracts ($\text{Log } K_{ow} > 2$), indicative of electronegative compounds like chlorinated substances, are much lower, and range up to 10 kg/y for individual effluents.

DR-CALUX loads: Figure 5.5 presents the summed DR-CALUX loads of all chlorine-chain effluents studied in the OVOC study, as based on total extracts, the sum of coarse fractions (low, medium, high $\text{Log } K_{ow}$ fraction), the sum of fine fractions (to

¹⁶ Based on a recent, comprehensive review of literature data on Ah-receptor agonists by Aarts and Palmer (2002).

the extent investigated) and the sum of fine fractions with acidic clean-up applied. Although not all of the available fine fractions were analysed ($\text{Log } K_{ow} < 4$ and 4-5 fractions were not included), little information was lost, as most of the response was observed in the fractions with $\text{Log } K_{ow} > 6$ (see Figure 5.1).

The calculated DR-CALUX load varies from 32.4 g bio-TEQ/y based on total extracts without acidic clean-up to 2.7 g bio-TEQ/y based on fine fractions after acidic clean-up. The contribution of substances that are not (or less easily) metabolisable or degradable by the acidic cleanup is apparently only about 5% of the initial DR-CALUX response. The total load thus decreases considerably in the course of fractionation and as a result of acidic clean-up. This is especially true for the response of effluent MCD¹⁷. Cooling water (2.1 g bio-TEQ/yr) and effluent MEF (0.6 g bio-TEQ/yr) dominate in the loads after acidic clean-up, with a minor contribution of effluent MCD (0.02 g bio-TEQ/yr).

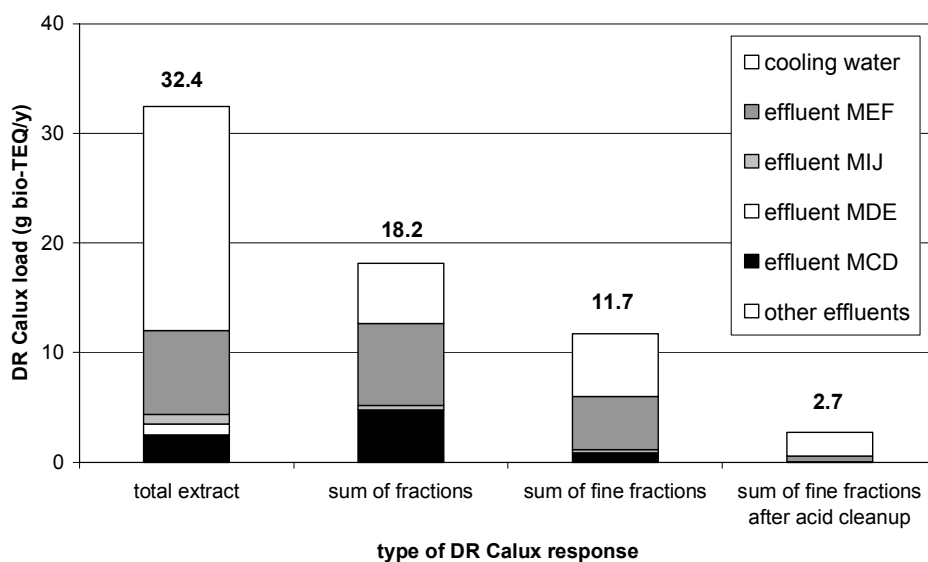


Figure 5.5 DR-CALUX loads (g bio-TEQ/yr), as determined for the chlorine-chain effluents in the OVOC study.

Note: For effluent MCD, only the estimated contribution from chlorine activities is given.

Source: Based on data in OVOC reports by Lamoree et al. (2003, 2004a) and by Senhorst et al. (2004b).

The DR-CALUX response in the total extract and the coarse fractions of the extract of sample MCD contain a significant contribution of non-chlorine-chain activities. Results of the in-plant TIE study (Appendix 9.7) suggest that approximately 10% of the MCD response in total extracts (without acidic clean-up) and fractions originate from chlorine activities. Figure 5.5 provides only the chlorine-chain contribution.

¹⁷ For MCD, the load based on total extracts also originates predominately from non-chlorine activities. In the load after acidic clean-up, however, only chlorine-chain emissions of MCD are present.

Table 5.3 Atmospheric emissions of chlorinated compounds detected in fractionated extracts of emission samples with GC-NCI-MS.

Sample	Compound	Concentration in $\mu\text{g}/\text{Nm}^3$	Flue-gas volume (Nm^3/h)	Annual load kg/yr
MSWI 270-01	monochlorophenol ¹	8 – 80	254.000	17 – 170
	hexachlorobenzene	0.2 – 2		0.3 – 3
vent gas 270-02	monochlorophenol ¹	3 – 30	14.000	0.3 – 3.5
	hexachlorobenzene	0.4 – 4		0.05 – 0.5
vent gas 270-04	monochlorophenol ¹	2 – 17	17.000	0.3 – 2.5
vent gas 270-05	pentachlorobenzene	2 – 7	2281	0.004 – 0.04
	hexachlorobenzene	0.2 – 3		0.01 – 0.13
	unknown Cl ₄	0.1 – 1		0.002 – 0.2

¹Refers to a substance with a monochlorophenol fragment.

Source: OVOC report Van Hattum et al. (2004a).

Emission loads into the air

Chlorinated substances: Table 5.3 presents calculated emission loads. The loads corresponding to sample 270-01 are relatively large, as they concern a solid waste incineration facility emitting correspondingly large flue-gas volumes.

DR-CALUX loads: Total DR-CALUX emissions from the three vent-gas installations (Table 5.4) were estimated to be 0.06 g bio-TEQ/yr based on the results of this survey; they seem small (< 2%) compared to recent estimates for atmospheric emissions of dioxins from all industrial sources in the Netherlands (3 g I-TEQ/yr). Extrapolation to all vent-gas emissions from the chlorine chain is not possible at this stage, as reliable data on the number and capacity of vent-gas incinerators in chlorine industry in the Netherlands are not available.

Loads via products

Chlorinated substances: The loads of chlorinated substances in products in the Dutch chlorine chain are somewhat more difficult to calculate, due to the lack of reliable non-confidential data on usage volumes of these products in the Netherlands. Table 5.5 provides estimates of annual loads based on the results of both the tentative identifications and indicative characterisations of impurities in procust (see Table 5.1).

It should be emphasized that the loads presented here refer to chlorinated impurities in product flows and cannot be interpreted as loads of chlorinated substances into the environment. Many different parameters, including usage patterns and emission routes, determine for each product which part of the impurity load is actually emitted into the environment.

Because of its solubility in water, hydrochloric acid is often used in many water-based processes. In addition to the HCl itself, impurities may also end up in wastewaters.

Table 5.4 Atmospheric emissions. Estimation of annual loads of dioxins determined with HRGCMS and total dioxin-like activity determined with DR-CALUX (including sulphuric acid clean-up).

Sample	Sum PCDD/ PCDF (g/y)	HRGCMS TEQs g I-TEQ/y	DR-CALUX based TEQs incl. acidic clean-up g bio-TEQ/y
MSWI 270-01	1.7	0.08	0.12
vent gas 270-02	0.5	0.03	0.04
vent gas 270-04	0.1	0.002	0.01
vent gas 270-05	1.0	0.003	0.01
Total chlorine chain emissions ¹	1.7	0.03	0.06

¹ Sum of three vent-gas emissions, excluding MSWI emission.

Source: OVOC report, Van Hattum et al. (2004a).

Table 5.5 Concentrations and estimated annual loads of chlorinated impurities identified or characterised in products.

Product	Usage t/yr ¹	Concentration range (mg/kg)	Estimated annual load kg/yr
CR	1000 ²	20-200	20-200
TRI	<500	0.3-3	<0.2-1.5
MCA	<10000	0.1-1.1	<1-11
AC	200	1.1-11	0.2-2.2
EDC	<80000	0.01-0.1	<0.8-8
PER	1477	0.003-0.03	0.004-0.04
HCl	<15000	0.01-0.1	<0.1-1.5
DCM	4050	<0.005	<0.02

¹ Excluding on-site captive use; ² Tentative estimate of annual usage.

Source: OVOC report, Van Hattum et al. (2004b).

For allylchloride, however, the case is very different. The majority of allylchloride is converted to epichlorohydrin that, in turn, is used as a building block in resins, for example in car paints. Impurities in allylchloride might then end up in the melt ovens of the secondary steel ovens. This creates a completely different dispersion pattern. Chloroprene rubber, as another example, is a solid material from which emissions during usage can occur only through leaching and weathering (if used under humid conditions). As the conditions during weathering are likely to be much milder than during the Soxhlet-extraction used in sample preparation (hexane/acetone mixture, 12 hr at 40 °C), the load emitted from chloroprene rubber during usage is likely to be much lower than the value reported in Table 5.5.

Additionally, because some products (e.g., TRI, PER, HCl) on the Dutch market originate from production processes other than the sources included in the current study, the profiles of impurities in a type of product are likely to differ strongly among products from the different suppliers on the national market. Further, extrapolations to the national market are therefore likely to be accompanied by a high level of uncertainty.

Table 5.6 Estimation of annual loads of bioassay-based bio-TEQs via selected products.

Product	Usage (tonnes/y) ¹	Estimated DR- CALUX load after acidic clean-up (mg bio-TEQ/y)	Worst-case esti- mated load for values < LOD (mg bio-TEQ/y)
dichloromethane (DCM)	4050		<0.04
1,2-dichloroethane (EDC)	<80000	<1.6 ²	
perchloroethylene (PER)	1500	0.06 ²	
trichloroethylene (TRI)	<500	<5	
allylchloride (AC)	200		<0.003
hydrochloric acid (HCl)	<15000	<30	
1,4-dichlorobenzene	50		<10 ³
monochloroacetic acid (MCA)	<10000		<0.3
Triclosan	3-16		<3-14 ³
chloroalkanes C ₁₄₋₁₇	1400		<28 ³
chloroprene rubber (CR)	1000 ⁴	1700	
Total		<1737	

¹ Usage excluding on-site captive use as intermediate in major production processes;

² Uncertain estimate based on response level between LOD and LOQ;

³ Worst-case estimate, due to limitation to highest concentration tested without cytotoxicity and extrapolation to tonnes/y;

⁴ Tentative estimate.

Source: OVOC report, Van Hattum et al. (2004b).

DR-CALUX loads: The estimates for the products (Van Hattum et al., 2004b) are indicated in Table 5.6. The load value given for chloroprene rubber (and thereby the total load from all products) should be considered tentative, as no reliable usage figures for chloroprene rubber were available. Chloroprene rubber clearly dominates the total DR-CALUX load (98%), with minor contributions from hydrochloric acid and trichloroethylene.

5.5 PBT character of identified chlorinated substances

Substances tentatively identified by automatic AMDIS/NIST analysis of GC-MS or GC-NCI-MS data in one or more of the studied extracts (wastewater, air and products) are shown in Table 5.7, together with information on their environmental characteristics:

- Persistency, as determined by the screening method in EU Interim PBT strategy, in which the BIOWIN estimation method is used to assess persistency (see Appendix 9.5);
- Bioaccumulation: the Log K_{ow} is reported as estimated from the KOWWIN module. This value can deviate by as much as ± 0.5 from experimental values;
- Occurrence on a number of international priority substance lists.

Persistency: The BIOWIN screening method identifies approximately half of the tentatively identified compounds as persistent, based on QSAR predictions and expert rules. Due to its purpose, the screening method is more likely to falsely attribute persistency to a substance than it is to overlook persistency (Traas et al, 2004).

Bioaccumulative properties: Of the 45 compounds on the list, 20 are classifiable as potentially bioaccumulative ($\text{Log } K_{ow} > 4$). Almost all of these compounds contain three or more chlorine atoms. This corresponds to the principle that, within classes of structurally closely related compounds, the $\text{Log } K_{ow}$ value increases with increasing molecular weight and/or number of chlorine atoms.

Presence on priority substance list: Of all tentatively identified compounds, nine appear on a priority substance list (Rhine Action Programme, OSPAR, EU or UN). Because the purpose of these lists is only to name the substances that are in most urgent need of attention, they contain only a very limited selection of hazardous substances. At present, several mechanisms have been developed in order for extending these lists. The absence of a substance from a priority substance list, therefore, does not imply that the substance is not harmful.

Extension to indicatively characterised chlorine-containing compounds

In the analyses of the product extracts and, to a lesser extent, of the extracts of effluents and air samples, a great number of GC-MS components were observed that could be interpreted as chlorine-containing substances based on the characteristic chlorine-isotope patterns. For some of these components, there were no hints concerning chemical structure. For many others, however, an indication could be given as to the possible chemical family. Very frequently, these indicatively characterised compounds appeared at higher retention times in the GC-MS (as in the case of trichloroethylene, see Figure 4.2) and had higher molecular weights (based on the highest observed ion mass) than did the compounds that could be identified by AMDIS. In the case of the products tri- and allylchloride, these indicatively characterised compounds were also more highly chlorinated than were the AMDIS identified compounds.

All of these characteristics (larger molecules, higher molecular weights, higher degrees of chlorination) tend to increase the $\text{Log } K_{ow}$. Additionally, these characteristics may contribute to a lower biodegradability (e.g., by steric hindrance, reduced bioavailability) and/or increase the chemical stability of a compound (e.g., if chlorine is added to aromatic rings). In other words, in comparison to the AMDIS identified compounds, many of the indicatively characterised chlorine-containing substances may be more persistent and/or have a higher bioaccumulative potency.

Table 5.7 Environmentally relevant characteristics of substances that were tentatively identified in atmospheric emissions and products of the chlorine chain in the Netherlands.

CAS ¹	Name ²	Occurrence in sample ³	Worst-case emission kg/y ⁴	Priority substance list ⁵	Log K _{ow} ⁶	Persistent according to BIOWIN ⁷
29480420	1,5-cyclooctadiene, 1,6-dichloro	neo	10-100		4.71	
13547063	cyclohexene, 1-chloro, 4-(1-chloroethenyl)	neo	1-10		4.72	
13547074	cyclohexene, 1-chloro, 5-(1-chloroethenyl)	neo	1-10		4.72	
5324685	benzene, 1,3,5-trichloro, 2,4,6-trimethyl	mca	0.1-1	yes	5.57	yes
4749273	1-propene, 3,3,3-trichloro, 2-methyl	neo	0.1-1		3.58	yes
1073672	benzene, 1-chloro, 4-ethenyl	neo	0.1-1		3.54	
104825	benzene, 1-(chloromethyl), 4-methyl	neo	0.1-1		3.34	
111911	ethane, 1,1'-methylene, bis(oxy) bis-2chloro	edc	0.08-0.8		1.3	
118741	benzene, hexachloro	01,02,05	0.06-0.6 ⁸	yes	5.86 (5.73)	
55880778	1,3-butadiene, pentachloro	tri	0.05-0.5		4.22	yes
42769380	1,3-butadiene, 1,1,3,4-tetrachloro	tri	0.05-0.5		3.73	yes
127184	ethene, tetrachloro	per,tri,dcm	0.015-0.15	yes	2.97 (3.4)	yes
54965207	benzene, trichloro-1,3-dimethyl-	hcl,mca	0.015-0.15	yes	?	?
54833135	benzene, 1,2,4-trichlorodiethyl-	mca	0.01-0.1	yes	?	?
877098	benzene, 1,2,3,5-tetrachloro, 4,6-dimethyl	mca	0.01-0.1		5.67	yes
3955268	benzene, 1,2,4-trichloro, 5-(chloromethyl)	mca	0.01-0.1		4.73	yes
54965014	benzene, 1,2-dichloro, 4-(1-chloroethyl)	mca	0.01-0.1	yes	4.5	yes
54411120	benzene, (2-chloro, 2-butenyl)	edc	0.008-0.08		4.29	
97972	ethane, 2-chloro, 1,1-dimethoxy	edc	0.008-0.08		0.48	
2568301	1,3-dioxolane, 2-(chloromethyl)	edc	0.008-0.08		0.36	
87683	1,3-butadiene, 1,1,2,3,4,4-hexachloro	per,tri	0.005-0.05	yes	4.72 (4.78)	yes
608935	benzene, pentachloro	tri,05	0.004-0.04	yes	5.22 (5.17)	yes

CAS ¹	Name ²	Occurrence in sample ³	Worst-case emission kg/y ⁴	Priority substance list ⁵	Log K _{ow} ⁶	Persistent according to BIOWIN ⁷
95501	benzene, 1,2-dichloro	dcm,tri	0.004-0.04	yes	3.28 (3.43)	
34973392	1-butene, 1,1,3,3,4,4-hexachloro	tri	0.0025-0.025		4.56	yes
87865	phenol, pentachloro	hcl	0.0015-0.015	yes	4.74 (5.12)	yes
67721	ethane,hexachloro	per	0.0015-0.015	yes	4.03 (4.14)	yes
88062	phenol, 2,4,6-trichloro	hcl	0.0015-0.015	yes	3.45 (3.69)	yes
1888717	1-propene, 1,1,2,3,3,3-hexachloro	per,tri	0.0015-0.015		4.38	yes
54965194	benzene, trichloroethyl-	mca	0.001-0.01	yes	?	?
74421515	methyl, E-2,3-dichloropropenoate	mca	0.001-0.01		?	?
6298722	benzene, 1,4-bis(chloromethyl), 2,5-dimethyl	mca	0.001-0.01		4.69	
2077465	benzene, 1,2,4-trichloro, 3-methyl	mca	0.001-0.01	yes	4.47	yes
111444	ethane, 1,1'oxybis 2chloro	edc	<8e-3		1.56 (1.29)	
5409756	ethane, 2-chloro-1,1-bis(2-chloroethoxy)-	edc	<8e-3		?	?
1123848	benzene, 1,4-dichloro, 2-ethenyl	edc	<8e-3		4.18	
2436739	acetic acid, (4-chloro, 2-methylphenoxy), methyl ester	edc	<8e-3		2.81	
2233003	1-propene, 3,3,3-trichloro-	dcm	<4e-3		3.03	yes
13116579	1-propene, 1,2,3-trichloro-,	ac,tri	<2e-3		?	?
594650	acetamide, 2,2,2-trichloro	per	<1.5e-3		0.83 (1.04)	yes
116541	acetic acid, dichloro, methyl ester	tri,edc	<8e-4		1.66	
56631017	butene, hexachloro	tri	<5e-4		?	?
20589859	1-propene, 1,2,3,3-tetrachloro	tri	<5e-4		2.96	yes
96184	propane, 1,2,3-trichloro	ac	<2e-4		2.5 (2.27)	
34632898	2,4-hexadiene, 1-chloro	ac	<2e-4		3.11	
96195	1-propene, 1,2,3-trichloro	ac	<2e-4		2.78	
691930	propanoic acid, 3-chloro, 1methylethyl ester	ac	<2e-4		2.03	

CAS ¹	Name ²	Occurrence in sample ³	Worst-case emission kg/y ⁴	Priority substance list ⁵	Log K _{ow} ⁶	Persistent according to BIOWIN ⁷
598992	acetic acid, trichloro, methyl ester	per,tri	<1.5e-4		1.73 (2.03)	
1871585	propane, 1,2,3-trichloro, 2-methyl	per	<1.5e-4		2.96	yes
89689	phenol, 4-chloro, 5-methyl, 2-(1-methylethyl)	tri	<5e-5		4.16 (3.92)	
76017	ethane, pentachloro	tri	<5e-5		3.11 (3.22)	yes
79345	ethane, 1,1,2,2-tetrachloro	tri	<5e-5	yes	2.19 (2.39)	yes
2346818	hexane, 3-chloro	tri	<5e-5		3.47	

¹ Five substances identified with 'EPA numbers' are not incorporated in this list, as no Log K_{ow} info was available;

² Name and CAS number are taken from the AMDIS output and should be considered as a tentative identification. Stereo-isomers or related family members could also be relevant;

³ Codes used refer to atmospheric emissions (01,02,05) and products (ac=allylchloride, dcm=dichloromethane, edc=1,2-dichloroethane, hcl=hydrochloric acid, mca=monochloroacetic acid, cr=chloroprene rubber, per=perchloroethylene, tri=trichloroethylene);

⁴ Based on estimated concentrations, as given in van Hattum 2004;

⁵ Based on a compilation of EU/ECB (REACH), OSPAR (possible concern and priority action lists), UN POPs, Rhine Action Program, 76/464 EG ('black' and 'grey' lists) and EU Water Framework Directive lists;

⁶ Values given refer to Log K_{ow} estimates, as calculated by the KOWIN software module. When present, experimental values appear in parantheses. For guidance in OVOC, the cut-off values of 4 and 6 are used for bioaccumulative and very bioaccumulative, respectively; ? = no computation possible; bold: Log K_{ow} >4

⁷ Cut-off criteria for persistency taken according to screening criteria in EU Interim PBT strategy: BIOWIN results are <0.5 for model 2 (nonlinear) or model 6 (Miti non-linear) and < 2,2 for model 3 (ultimate biogradation); ?= no computation possible;

⁸ Value determined by atmospheric emission.

General picture

The picture that now emerges is that many of the tentatively identified compounds can be characterised as substances with possible PB properties. The toxicity characteristics may differ from compound to compound. Collection of toxicity data for these compounds was beyond the scope of the project. For compounds with a $\text{Log } K_{ow} > 6$, the non-specific baseline toxicity mechanism (narcosis) may become relevant (see toxicity criterion of the EU interim PBT strategy). Although nothing can be said specifically on toxicity unless the substance in question has been properly identified, the baseline toxicity by itself may be significant for high $\text{Log } K_{ow}$ substances. In the proposed EU-REACH and Dutch SOMS strategy, very persistent and very bioaccumulative substances (vPvB) are considered as a separate class, and no additional toxicity criterion must be fulfilled (see Appendix 9.5).

The indicatively characterised compounds, many of which are assumed to be more persistent and bioaccumulative than are the tentatively identified compounds, may therefore be more harmful to the environment. In summary, it is possible to say that a substantial number of the chlorinated substances that have been identified as possibly present in the products and vent-gas emissions can be considered to have potential PBT properties.

Although the substances may be problematic for the environment, the *amounts* in which they may be emitted into the environment are very limited, compared to known emissions of these or related substances. For a number of chlorinated substances, emissions to the municipal sewer system are known for the Netherlands (*Emissiemonitor* 2000, 2002). Dispersed emissions from products to wastewater are often also discharged to municipal wastewater treatment facilities; these values can therefore be compared to the worst-case emission estimates for products, as shown in Table 5.7. For atmospheric emissions, a comparison can be made to data for the Netherlands from a recent OSPAR compilation (OSPAR, 2002).

For five substances identified by OVOC a direct comparison with known emissions is possible. Table 5.8 presents the results and shows that, for three of these substances (pentachlorophenol, trichlorobenzene and perchloroethylene), the worst-case load is much lower than the corresponding emission value (wastewater or air). For hexachlorobenzene, however, known air emissions are registered as zero, while for hexachlorobutadiene, no emission values to sewer systems are known.

Individual wastewater emissions of chlorinated substances (except hexachlorobutadiene) listed in Table 5.8 and classified by OSPAR as hazardous vary between 15 and 3000 kg/y. The total load of all these substances to wastewater is almost 3300 kg/y. This load is an order of magnitude larger than the total worst-case maximum load for emissions of chlorinated substances via products (including indicatively identified substances this would be somewhat more than 200 kg/y¹⁸; see Table 5.5).

¹⁸ This load is dominated by emissions from chloroprene rubber. The actual emissions are expected to be much lower than this worst-case emission value (Section 5.4).

Table 5.8 Comparison of OVOC-identified worst-case emissions with known emissions in the Netherlands to municipal sewer systems or air.

Chlorinated hazardous substance	Emissions to municipal sewer systems 2000 ¹ kg/y	Atmospheric emissions 1999 ² kg/y	OVOC-identified worst-case load ³ kg/y
pentachlorophenol	92	25000	0.015 ⁴
hexachlorobenzene	15,6	0	0.6 ⁴
hexachlorobutadiene	n.a.	n.a.	0.05
perchloroethylene	120	2x10 ⁶	0.15
trichlorobenzene	3050	194	1.26 ⁵

¹ Data from *Emissiemonitor* 2000; n.a. = not available;

² Data from OSPAR compilation; n.a.= not available;

³ Upper maximum of load in Table 5.7;

⁴ Refers to atmospheric emission;

⁵ Sum of several (alkylated) isomers.

Even if we were to classify all chlorinated impurities in products as hazardous, and even if we did not take into account that only a part of these substances will be emitted from the products, their total load constitutes only a limited contribution to the known load of the five chlorinated hazardous substances. The load of chlorinated impurities identified by OVOC therefore seems to be of limited importance for exposure levels on a national scale.

5.6 Discussion of *in vivo* results

No toxicity found in zebra fish ELS test

A highly significant result of the *in vivo* tests on the effluent extracts is the absence of a response in the zebra fish ELS test. A question then arises concerning whether the effluents are not toxic to fish at all. A separate study analysed the information on toxicity of industrial effluents, which was available at the Institute for Inland Water Management and Wastewater Treatment (RIZA) of the Netherlands (Gerritsen, 2004). In this analysis, fish toxicity data based on whole effluent analysis (WEA) for two of the effluents studied in OVOC were available at RIZA. Both effluents proved to be toxic. This difference in results may be explained by the L/L extraction pre-treatment applied in the OVOC study, allowing only the more hydrophobic substances ($\text{Log } K_{ow} > \pm 2$) to be present in the test. We can therefore conclude that the absence of a toxic response in extracts does not necessarily imply the absence of a toxic response in the 'whole' effluent. This may further indicate that, in the whole effluent studies, more polar or ionic compounds could have been responsible for the induction of effects.

Assessment with chronic Microtox test

Of the 21 samples tested with the chronic Microtox test in OVOC, seven samples proved to be toxic. The most toxic sample of these lost its response at 12-fold dilution with respect to the effluent concentration. This level of toxicity is comparable to the levels

found in the RIZA analysis for non-chlorine-chain effluents¹⁹. The level of Microtox found in the OVOC study is thus comparable to toxicity levels found in non-chlorine-chain industrial effluents.

The fact that the extracts showed a response in the chronic Microtox test and not in the ELS test indicates that the fish ELS test is less sensitive for the extracted compounds than the Microtox test, which is in line with observations from literature (see also the description of the chronic Microtox test presented in Section 4.3).

Comparison of chlorine-chain effluents with other industrial effluents

The hypothesis has been tested that chlorine chain effluents are comparable in *in vivo* toxicity to non-chlorine chain effluents²⁰. Of the five toxicity tests used in the comparison with RIZA toxicity results, only one test (crustaceans) gives a significantly higher frequency of toxicity among chlorine-chain effluents than among non-chlorine-chain effluents. Because no good parameter is available for testing the comparability of (groups of) effluents, this result should be interpreted with caution. For instance, when all municipal wastewater treatment plants are removed from the group of non-chlorine-chain effluents (leaving only industrial effluents in the group), the difference in toxicity between chlorine-chain effluents and non-chlorine-chain effluents disappears. The hypothesis can therefore not be rejected on the basis of the information available at RIZA. The overall conclusion is therefore that, in terms of toxicity to bacteria, algae, crustaceans and fish, the chlorine-chain effluents are no different from non-chlorine-chain industrial effluents.

5.7 Discussion of *in vitro* results

The wastewater studies (Lamoree et al., 2003) and the biodegradation studies (Senhorst et al., 2004a) revealed a reasonable correlation between the DR-CALUX response and the EROD induction in the Carp-hep assay. All Carp-hep EROD responsive samples were also responsive in the DR-CALUX test. The relative ranking of the response was different in both tests and some DR-CALUX responsive samples were not responsive in the EROD test. This is in line with findings from other comparative studies (Sanderson et al, 1996), and probably reflects compound-specific differences in sensitivity between mammalian and fish liver cells; it probably also reflects a lower influence of transient inducers in the 96-h Carp-hep tests and the lower limits of detection of the DR-CALUX system. The concurrence of the messenger RNA activation in the mammalian cell (DR-CALUX response) and the EROD induction in the carp celline, indicates that, in both tests, specific subcellular responses of the Ah-receptor-mediated dioxin-like toxicity mechanism are activated upon exposure to wastewater extracts. This concurrence was observed in total extracts without clean-up, and it supports the interpretation that the assay results are consistent with dioxin-like toxicity at the subcellular level.

¹⁹ A 12-fold dilution is expressed as 12 Toxic Units (Tus). In the RIZA analysis, values of non-chlorine-chain industrial effluents of up to TU=30 were found.

²⁰ The long list of chlorine-chain emissions, as reported in Appendix 9.1.3, was used for the selection of chlorine-chain effluents.

Although anti-estrogenicity was observed in the Carp-hep test in some wastewater samples, it may have been related to the presence of dioxin-like compounds, some of which are known to have an anti-estrogenic potency (Smeets et al, 1999). The results of the anti-estrogenicity tests have therefore been omitted from the discussion.

An important question to address concerns the extent to which the results of the DR-CALUX test are indicative of the presence of chlorinated PBT compounds. A clear answer appears only in the case of atmospheric emissions, in which responses after acidic clean-up in three of the four samples were largely attributable to the measured PCDD/F concentrations. Only a limited portion of the response for the products and wastewater was attributable to measured PCDD/F concentrations: 5-21% for products and <1 % for wastewater effluent. Observations from the fractionation studies with effluents and vent-gas extracts showed that the highest response was found in medium and high Log K_{ow} fractions (Figure 5.2), indicating that the unexplained part of the response is related to unknown agonists with probable bioaccumulative properties.

The biodegradation experiments with wastewater effluents were inconclusive for the attribution of the persistency of the response, and they delivered no additional quantitative information. From the results of the DR-CALUX tests with acidic cleanup and with fractionated extracts, only some qualitative observations are possible. As discussed in previous sections, further fractionation of samples may have increased the importance of biotransformation in the 24-hour DR-CALUX test and decreased the influence of transiently inducing compounds. In this way, fractionation may contribute to further focussing on more persistent compounds. An alternate hypothesis to explain the decreased response in fractionated samples could be the loss of compounds involved in synergistic interactions. As most evidence from experiments with mixtures of congeners of dioxins and dioxin-like compounds seems to corroborate the concept of additivity, which is the basis of the TEF approach (Van den Berg et al., 1998), this explanation seems unlikely. The design of the current study, however, does not allow the full exclusion of this alternative explanation.

The validation studies of the acidic clean-up step (Lamoree et al., 2004b) indicated that, although this procedure is effective in removing most of the known responsive parent PAHs from the EPA set of 16 PAHs, the results after clean-up cannot be used as an indication of persistency. Some known (bio)degradable compounds are apparently not removed during the clean-up. On the other hand, some known persistent chlorinated compounds were removed during clean-up. As demonstrated in the biodegradation studies (Senhorst et al., 2004a) and the in-plant TIE study (Senhorst et al, 2004b) the acidic clean-up was effective in removing sometimes-high concentrations of interfering (probably transient) inducers. In this respect the DR-CALUX response after refined fractionation and acid clean-up, may be interpreted as acid-stable compounds that are not or poorly metabolised in mammalian liver cells during the 24-hour duration of the test.

There is no single scale on which to measure persistency, as persistency results from a number of processes in both the environment and organisms (abiotic degradation, biodegradation, biotransformation). Nevertheless, Figure 5.6 illustrates the placement of such terms as PBTs and dioxin-like compounds within the framework of the environmental assessment of OVOC (focussing on PBTs and potential food-chain effects).

It presumes the existence of a hypothetical, ordinal scale of persistency and shows how the DR-CALUX response of extracts and fractions can be ranked on this scale. With respect to the DR-CALUX response, both fractionation (via metabolism) and application of acidic clean-up contribute to the removal of transient inducers, and this results in a shift to the right in Figure 5.6.

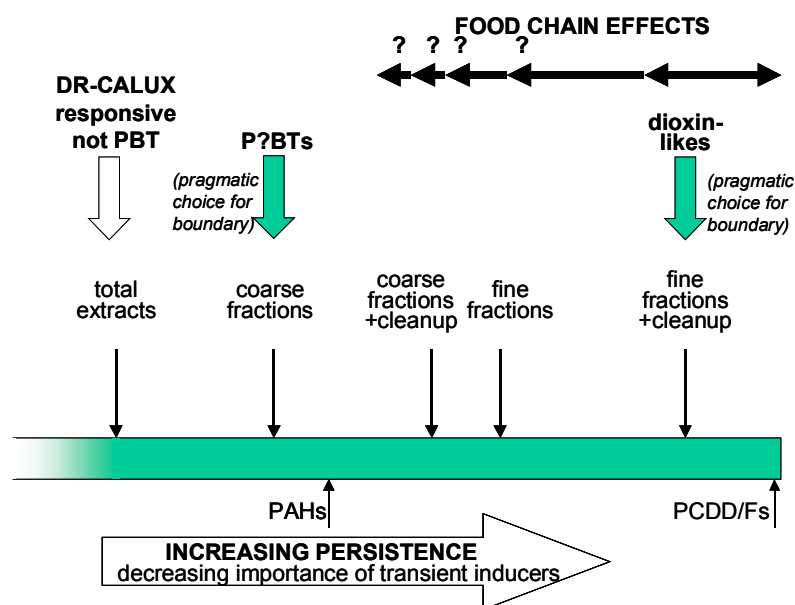


Figure 5.6 Conceptual scheme of qualitative ranking of the DR-CALUX response in samples after refined fractionation (determinations in fractions with medium and high hydrophobicity) and after acidic clean-up.

Within this conceptual scheme, the response of the fine fractions of the wastewater samples after acidic clean-up represent the most persistent form of DR-CALUX response. As we also know, the inducing substances are probably bioaccumulative and express toxicity at least at the subcellular level (see above), and they have properties resembling dioxin-like compounds (Section 3.3). For the purpose of the environmental assessment given in Chapter 6, therefore, this response will be pragmatically attributed in a worst-case approximation to dioxin-like compounds. There is a distinct probability that inducing substances in this signal are not yet persistent enough to be considered dioxin-like, but for simplification this attribution allows a worst-case quantification of the presence of dioxin-like compounds and the comparison of emission loads to known dioxin emissions.

The focus of OVOC, however, is not strictly on dioxin-like compounds, but rather on substances with PBT character (see Section 1.2) that are likely to induce food-chain effects. In an attempt comparable to that conducted for dioxin-like compounds, the response in the coarse fractions without clean-up is assumed to have PBT character to some extent in order to characterise the observed DR-CALUX response observed:

- Persistency: substances have survived at least 24-hour metabolism in the liver test cell, and often have already passed biological wastewater treatment (except for cooling water and effluent MEF in Figure 5.5);

- Bioaccumulation: all responses in the coarse fractions are concentrated at $\text{Log } K_{ow} = 4$ or higher;
- Toxicity: because of the concurrence of DR-CALUX response and EROD signal dioxin-like toxicity is probable. The Carp-hep test is less sensitive to transient inducers.

This attribution of the DR-CALUX response to PBT substances is not strict; the $\text{Log } K_{ow}$ boundaries of the coarse fractions are not sharply defined. Feedback and repair mechanisms may preclude the expression of the DR-CALUX response in organisms, and, most of all, 24-hour metabolisation in test cells bears no (quantifiable) relation with respect to persistency. Nevertheless, the response in coarse fractions without clean-up will be assumed to have PBT character, in order to give an upper limit to the part of the DR-CALUX response that may be relevant in the environmental assessment of Chapter 6.

Part 3 Synthesis and Conclusions

6. Synthesis

This chapter discusses the experimental results presented above against the background of the two objectives of OVOC, as presented in Section 1.2. First, the experimental data are presented *as measured*, leading to an overall conclusion in answer to the objectives. The methodological limitations with respect to this conclusion are then addressed, looking beyond the actual measurements to the robustness of the conclusion. Finally, the results are discussed in the light of an evaluation of recent emission-reduction programmes (Tukker, 2004), and a brief summary is provided of the remaining uncertainties in the overall results and conclusions.

6.1 Addressing the goals of the OVOC project

The two goals of OVOC are:

- *To assess whether and to what extent PBTs or groups of PBTs are emitted from the chlorine chain into the environment;*
- *To contribute to the assessment of the environmental impact of these PBTs.*

From these two goals, three questions can be derived that will subsequently be addressed in the course of the text:

- a) Are substances with PBT properties produced in the industrial production processes of the chlorine chain? If so, to what extent?*
- b) Are substances with PBT properties emitted from the chlorine chain processes into the environment? If so, to what extent?*
- c) What are the environmental hazards of possible PBT emissions?*

- a) Are substances with PBT properties being produced in the industrial production processes of the chlorine chain? If so, to what extent?*

Yes. Substances with PBT properties are present and probably formed in the industrial processes of the chlorine chain. This answer is based on the observation that a number of products contain PCDD/Fs (Table 5.2) or chlorinated impurities that can be characterised as having PBT properties (see Section 5.7). In addition, PCDD/Fs were observed in one wastewater effluent. The nature of the observed impurities (i.e., the chemical properties and thereby the physico-chemical behaviour of these substances in the environment) varies strongly from product to product, however, as does the content of these impurities. The formation of PBTs and its extent are therefore not general characteristics of chlorine-chain processes. Consequently, they can be evaluated only on a process-to-process base.

By-product management

By-products are formed in any complex industrial chemical process. In general, industrial processes are designed to deal with by-product formation, mainly through the application of conditions that prevent or reduce the formation of unwanted by-products, and through the application of product purification steps. For many products, this purification

consists of distilling the crude process reactant, removing lower boiling fractions through the evaporation of gases and removing higher boiling fractions by removing the bottom residue. At present, most of these residue flows in the chlorine-processing industry are treated by incineration (gaseous residues, solid waste and hazardous aqueous waste) and/or wastewater treatment (non-hazardous aqueous waste). Figure 6.1 provides a layout of such an optimised industrial process. Emissions to the environment can occur by way of atmospheric emissions, discharged wastewater or dispersed emissions that result from product use.

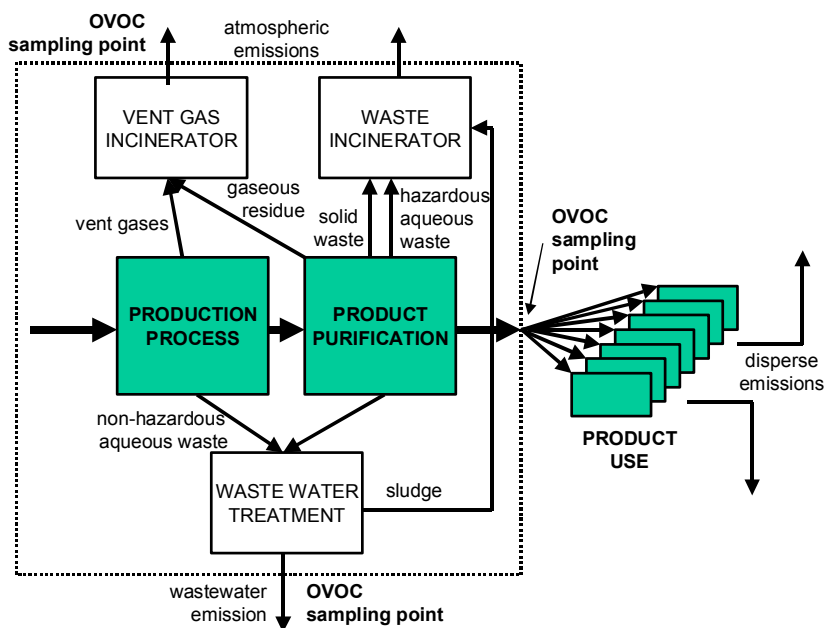


Figure 6.1 General layout of an industrial production process in the chlorine industry, indicating the various ways in which by-products that are formed are removed from process streams such that minimal emissions to the environment occur.

b) Are substances with PBT properties emitted from the chlorine chain processes?
If so, to what extent?

Yes. PBT substances are emitted from chlorine-chain processes, at least through the use of products in which they have been detected. A few chlorinated PBTs and PCDD/Fs were found in vent-gas emissions and some products, and PCDD/Fs were present in one effluent. Moreover, compounds with possible PB properties that were present in various products may lead to emissions.

Although in wastewater effluents, chlorinated compounds have been quantified only in the forms of AOX and EOX, the results described above indicated that these parameters consisted mostly of substances that were either volatile or more polar. Bioaccumulation is therefore less relevant here. GC-ECD-based measurements of total chlorinated organic compounds in pentane extracts indicated much lower concentrations (0.2-30 µg/L) with the largest contribution in fractions with low hydrophobicity. No chlorinated compounds

were identified with GC-MS or GC-NCI-MS above the limits of detection in the high Log K_{ow} fractions of effluents. This does not necessarily imply the absence of these compounds, as identification is limited and has a negative bias towards larger and more complex substances that have higher Log K_{ow} values. In addition, the detectability of by-products in effluents may greatly be reduced, due to the large dilution factors by which wastewaters of separate production units are mixed into the final effluent that is discharged into the environment²¹.

The conclusion that substances with PBT properties are emitted by way of products should be restricted in the sense that the magnitude of emissions depends on specific patterns of usage and specific waste treatment processes relevant to each usage pattern. The probability that impurities will be emitted thus differs from product to product, but it cannot be fully excluded.

c) What are the environmental hazards of possible PBT emissions?

The following discussion of potential environmental hazards distinguishes between direct effects on organisms and indirect effects that may be expressed on the level of the food chain. The potential induction of direct effects can be derived from the *in vivo* tests on aquatic effluents. Food-chain effects (secondary poisoning) pertain to toxic effects induced by bioaccumulative and persistent substances. The DR-CALUX response is used as an indicator for this type of effect, similar to the current approach for evaluating harbour dredgings (Stronkhorst *et al.*, 2002). The two types of effects should be separated: when emissions do not lead to direct effects, there may still be a risk for food chain effects.

Direct effects: The direct effects of the effluents studied do not appear to indicate exceptionally high environmental hazards. The fish ELS test shows no direct effects at all. Although the chronic Microtox test does show some response, the magnitude and scale of occurrence of this response was similar to other industrial effluents (Section 5.6).

Food-chain effects: Estimating potential food-chain effects can only be done in this study based on an evaluation of emitted loads, rather than concentrations or response levels. The emitted loads of identified substances with possible PBT character are relatively low, compared to known emissions of substances of similar character (Section 5.5) and are not expected to increase exposure levels significantly on a national scale, in comparison to contributions from known emissions from other sources.

In a worst-case approach (as discussed in Section 5.7), the DR-CALUX-based loads of bio-TEQs are considered equivalent to emissions of real dioxins and dioxin-like compounds in order to assess their potential contribution to possible food-chain effects. Table 6.1 compares registered emissions of dioxins (PCDD/Fs) in the Netherlands, derived from recent emission inventory studies (see Section 2.3) with the results of the current study.

²¹ The larger production sites studied (e.g., MEF, MCD) consist of dozens of production units. The wastewater from each unit often undergoes pretreatment to remove specific pollutants. The wastewater from all production units is subsequently brought together and discharged, after collective wastewater treatment, if available. Wastewaters may thus be diluted by a factor that is estimated for a number of sites to be between 10 and 1000.

Table 6.1 Comparison of DR-CALUX-based annual loads (after acidic clean-up and/or fractionation; in g bio-TEQ/yr) for selected sources in the OVOC project with data from recent dioxin-emission inventories (in g I-TEQ/yr).¹

Source category	Recent dioxin inventories				Contributions from OVOC project	
	RIVM 1999 RIZA Review database		Recent studies reviewed in De Koning (2004)		Source	Worst-case DR-CALUX-based load
	I-TEQ		I-TEQ			
	year	g/yr	year	g/yr	g/yr	
<i>Waste management</i>						
• air	1999	4	2000	10		
• water	1999	0.65	2000	0.024		
• land			1994	127-822		
<i>Industrial</i>						
• air	1999	3	2000	43-58	Vent gas	0.06
• water	1999	0.17	2000	0.04	Effluents	0.6
• land			1994	17-170	Cooling water	2.1
• disperse					Products	< 1.7
<i>Non-industrial</i>						
• air	1999	28	2000	13-14		
• water	1999	0.4-2	2000	0		
• land			1994	2.3-768		
Total including land emissions				220-1840		
Total without land emissions		37-38		66 - 82		< 4.4

¹ Emission data dioxins: see Table 2.3.

How should the environmental hazards of the DR-CALUX loads attributed to dioxin-like compounds be assessed?

- *Atmospheric emissions:* Vent-gas emissions have yet to be monitored. The measured load therefore forms an extra emission that can be added directly to the dioxin mass balance. The magnitude of this load (0.06 g bio-TEQ/y for the three installations studied) is insignificant (<0.1 %), however, compared to known atmospheric dioxin emissions (approximately 35-82 g I-TEQ/y).
- *Product emissions:* As already discussed, the emission loads calculated for product emissions should be interpreted as worst-case estimates. This is certainly true for the DR-CALUX load, as it is dominated by the contribution from chloroprene rubber. Polychloroprene is used in such products as paints, floor mats and diving suits. Leaching of impurities in these applications yields DR-CALUX loads that are lower than those indicated in Table 6.1²². In addition, the DR-CALUX response in products was determined in total extracts. As worst-case estimates, therefore, the inducing

²² By direct contact with the skin, however, leaching from diving suits may lead to significant human exposure.

- substances cannot be characterised as dioxin-like compounds, as in the approach followed for wastewater. Nevertheless, even within this worst-case assessment, the load from products (1.7 g bio-TEQ/y) may make only a minor contribution (2-5%) to the direct emissions of dioxins to water and air (approximately 37-82 g I-TEQ/y).
- *Effluents*: The worst-case estimate from the DR-CALUX test results for the load of possible dioxin-like compounds (2,7 g bio-TEQ/y) is an order of magnitude larger than known dioxin emissions from industrial effluents (0.04 – 0.17 g I-TEQ/y). Nevertheless, whether this will have a large effect on expected surface water concentrations is debatable. Some atmospheric emissions will end up in surface waters and, because of the magnitude of dioxin emissions to air, this may represent a considerable additional load to surface waters. The emissions of DR-CALUX-responsive compounds to surface waters may, nevertheless, give rise to locally increased hazards, depending on the specific discharge situation. Compared to the total direct emissions to water and air (37-82 pg I-TEQ/yr), the worst-case contribution of effluents from the chlorine chain is 3-7%.

Based on the above considerations, we conclude that the total DR-CALUX loads determined in this study (< 4.4 g bio-TEQ/yr), even considered in a worst-case approach as comparable to emissions of dioxins, constitute a limited (<5-11%) contribution to the total direct emissions of dioxins to water and air (37-82 g I-TEQ/yr) in the Netherlands. The direct emissions from vent-gas treatment installations seem insignificant (<0.1%).

The estimated contributions from the current study are far less than those from other sources of PCDD/Fs, such as potential emissions to land (200-1800 g I-TEQ/yr), or sources for which no reliable data are available, such as remobilisation of historic emissions from sinks (sediments, soil) and transboundary inputs (water, air). Acknowledging the large uncertainties involved in estimating annual loads, the results indicate that the current direct emissions from the chlorine chain probably represent only a minor contribution to current national environmental exposure levels of dioxins and dioxin-like compounds.

Comparison with dioxin emissions is not the only relevant contribution to the environmental assessment of chlorine-chain emissions, however. Food-chain effects are obvious for such highly persistent substances as dioxins and dioxin-like compounds. Less persistent transient inducers that still can be characterised as PBTs, however, may also be of possible significance for environmental assessment. The worst-case attribution of the DR-CALUX load to these “BT” substances with limited persistency (Section 5.9: based on coarse fractions without clean-up, a total load of 18.2 g DR-CALUX-based TEQ/y is computed) is a factor of 6 larger than the worst-case load attributed to dioxin-like compounds (2.7 g bio-TEQ/y). Most of the transient inducing substances, however, are prone to such degrading mechanisms as metabolism (in organisms) or biodegradation (in the environment), and they will therefore contribute mainly to non-persisting effects. The degree by which they may contribute to food-chain effects depends on many different properties, including bioavailability, biodegradation, bioaccumulation, biotransformation, food-chain transfer and the likelihood of the expression of toxic effects. Evaluation of these aspects is not possible without more information on the identity of the responsive compounds, and was beyond the scope of this study. Nevertheless, it does not seem likely that the additional hazards of these transient inducers are significant in comparison to the estimations of the worst-case DR-CALUX load (after acidic clean-up) attributed to

dioxin-like compounds. These hazards of transient inducers have therefore not been incorporated into the environmental assessment here.

Although the environmental hazards of current chlorine-chain-related emissions of compounds with PBT properties on a national scale appear to be relatively limited, local effects may still be relevant for a limited number of effluents. These local effects do not pertain to direct effects (as measured by the *in vivo* tests), but they may be relevant for some of the EROD- and DR-CALUX-responsive compounds. The extent to which local effects are relevant strongly depends on the local hydrodynamic exchange conditions at the point of discharge. When effluents are not directly mixed into large, high-exchange water systems, local effects might be present due to steady and gradual accumulation in sediments or soil.

Another issue might be the possible leaching of impurities from products that are applied in consumer products, such as chloroprene rubber (e.g., diving suits) and possibly triclosan (disinfectant used in tooth paste, for example). The chloroprene rubber tested was shown to contain high levels of chlorinated and DR-CALUX-inducing substances. The findings of this study may suggest a previously unknown pathway for human exposure to DR-CALUX-responsive compounds. Additional information on the causative agents, representative exposure conditions, and differences in the technical qualities of chloroprene is required, however, before further conclusions on the significance in this respect can be drawn.

Overall conclusion:

Although substances with PBT properties are present and probably produced as by-products in chlorine-chain production processes, the nature and amounts of these by-products cannot be generalised, and they vary from process to process. Apparently because of the effectiveness of present treatment systems for waste, atmospheric emissions and effluents, the estimated emissions of chlorine-chain processes constitute a minor contribution, in comparison to the present dioxin mass balance. The direct emissions of vent-gas treatment installations seem insignificant in this respect.

6.2 Methodological limitations relevant for conclusion

The conclusion presented above was based on the experimental results of this study and the data *as measured*. Because of methodological choices made in the design of the experimental framework and the constraints of each experiment, the conclusion that only limited emissions and effects are likely to occur should be interpreted in light of the methodological limitations inherent in the design of the study.

First, the data as measured do not reflect temporal variability, although care was taken to execute sampling under normal process conditions. Variations in emissions by fluctuations in emission composition were not taken into account. Especially for atmospheric and aquatic emissions, this may lead to a significant margin of uncertainty in the loads that were determined. For one effluent in this study, some information was obtained on the magnitude of this fluctuation. For effluent MCD, a difference between two independent samples (collected in 2001 and 2002 respectively) of a factor of six was observed for the DR-CALUX response in the coarse fractions after acidic clean-up. Apparently, fluctuations of this order can occur.

Second, the DR-CALUX load of effluents was dominated by assumed emissions from cooling water systems applying hypochlorite as biocide. The corresponding value was determined from one sample at one cooling-water installation that had a high dosage of hypochlorite, and was extrapolated to all such cooling systems in the Netherlands. This extrapolation to the national level was based on hypochlorite consumption, assuming a linear relationship between consumption and DR-CALUX activity. Although this strategy may appear legitimate in the absence of relevant data, we have no information that validates this extrapolation. This should be considered as an additional uncertainty inherent in the cooling-water loads calculated.

With respect to the potential emissions of impurities from products, it should be noted that the estimated load values are highly uncertain, as national-use data on non-captive usage were not available for all products (some figures were confidential, the figure for CR is a rough estimate). Additionally, the figures presented should be considered only as worst-case estimates for emission loads into the environment by way of products. Actual emission loads depend on consumption patterns, emission routes, or waste-handling practices and may be much lower. Some products (e.g., TRI, PER, HCl) on the Dutch market originate from production processes other than those included in the current study. The profiles of impurities in particular products are likely to differ strongly among suppliers on the national market; extrapolations starting from a product of a selected single source to the total national market of that product will therefore involve a high level of uncertainty.

Because of its mission to look for unknown and/or unanticipated chlorinated PBTs, this study followed a screening methodology. This involves two specific limitations that may be relevant for the conclusion of this study:

1. There is a probability of *false positive* attribution of substances and effects. Closer, in-depth examination of the samples and extracts used in this study may show that certain characteristics were assigned in error to some substances or effects. The occurrence of false positives is an inherent limitation of screening studies and was accepted in the design of the experimental framework. False positives lead to an over-estimation of emissions and effects.
2. There is also a probability of *false negative* identifications in the GC-MS screening. False negative identifications may cause some substances to be overlooked. Because of the absence of dedicated sample treatment, there is a considerable chance that especially those types of substances that are present at low concentrations and that are more difficult to detect will be overlooked (Section 3.4). For example, this holds for PCBs, PCDD/Fs, PCNs and PAHs that require specific sample pre-treatment. Non-identifications, therefore, do not necessarily imply the complete absence of the corresponding substances.

Another limitation in the GC-MS screening, as demonstrated in the experimental studies, is that current public databases of mass spectra (e.g., the NIST database) are not sufficiently comprehensive to handle the extremely complex mixtures of by-products and degradation products (at the ppb-ppm concentration level) observed in effluents, waste streams and products. The NIST database contains contributions from researchers from all over the world in order to build a publicly accessible, reference database. Despite the vast number of entries in the database (> 100.000 of substances), the experimental work

in this study has shown that the NIST database has a positive bias towards smaller, less complex molecules. Especially when trying to identify reaction by-products (as in the product study) this provides a distinct limitation towards identification. Because of these limitations, the identification of compounds responsive in toxicity tests – one of the original objectives of this study (Figure 3.2) – could not be realised. Any study looking for unknown or unanticipated substances would probably face the same limitations with regard to the identification of responsive compounds.

The experimental studies showed that volatile substances were lost during fractionation (Lamoree et al., 2003). In addition, less hydrophobic substances were not transferred to the pentane extracts ($\text{Log } K_{ow} < 2$). We do not think that this phenomenon poses a serious limitations to the conclusions stated above, as the less hydrophobic substances in general are not considered relevant for food-chain effects. Additionally, for extremely hydrophobic compounds ($\text{Log } K_{ow} > 8$), the pentane-based extraction may not have been fully quantitative, leading to a slight (not quantified in this study) underestimation for this category of compounds.

The limitations discussed above, and the remarks made on false positives and negatives in the *in vitro* studies (Section 4.4) all contribute to a specific margin of uncertainty for the experimental results of this study. At issue is whether this uncertainty margin calls for a different overall conclusion of this study. That is unlikely. The conclusions stated above were based on two quantitative *worst-case* evaluations:

1. The emission loads associated with product impurities and atmospheric emissions with possible PBT character (Section 5.5): these loads were shown to be two orders of magnitude below present emissions of comparable substances on a national scale.
2. The DR-CALUX load assumed in a worst-case estimate as being similar to dioxins was compared to the present total direct emission of dioxins to water and air in the Netherlands, which was an order of magnitude larger (Section 6.1).

We consider the probability that, due to the uncertainties mentioned above, the *actual* loads may be one or more orders of magnitude larger than the *worst-case* estimates to be quite low.

6.3 Findings in light of recent emission reduction programmes

As indicated in Section 6.1, the conclusion of this study is that, although PBT substances are produced in varying degrees within the chlorine chain, by-product management effectively prevents serious emissions from occurring in the environment.

The historical study into measures taken by companies in the chlorine chain to prevent the emission of by-products helped to explain this conclusion (Tukker, 2004). For the three main chlorine sites in the Netherlands (Akzo Nobel Botlek, Shell Chemie Pernis and Akzo Nobel Delfzijl), an overview was made of the measures taken by companies involved since the mid-1980s to prevent the emission of (by-)products (Tukker 2004). The focus on these three locations was based on the fact that these are ‘home’ for most of the production processes that were a priority with regard to emissions to air and/or water and to the ‘suspect’ products attributed in the Chlorine Chain Study. For a detailed discussion, we refer to Tukker (2004). In broad lines, the study showed:

- *In plant-measures*: In many production plants, better process control, the use of more pure raw materials and other measures considerably reduced the production of by-products;
- *Emissions to air*: For virtually all plants covered here, emissions to air are treated by vent-gas incinerators. In principle, these incinerators should destroy any main chlorinated emissions to air, as well as any micropollutants that may be present. It is therefore conceivable that end-of-pipe measurements taken during incinerator operation detect only limited emissions of DR-CALUX activity;
- *Emissions to water*: Emissions to water have received considerable attention over the last 15 years and have been reduced by in-plant measures and/or by the addition of (advanced) biological and physical wastewater treatment plants. In addition, it is conceivable that low levels of DR-CALUX activity are recorded in end-of-pipe measurements.

The situation described in Tukker (2004) holds only for the actual situation in the Netherlands. In other countries, levels of environmental protection and waste treatment practices may be different, implying that a level of quality in emission control similar to that in the Netherlands cannot be assumed from the start. The conclusion of this study can therefore not be generalised to a larger geographical scale.

6.4 Remaining uncertainties concerning by-product management

In addition to the methodological uncertainties presented in Section 6.2, a number of uncertainties relate to the effectiveness of the by-product management scheme discussed above. All samples in this study were taken in normal production situations. This raises the question of the extent to which a production situation can be characterised as normal and how this may affect emissions and their effects. We have no quantitative information on this. We can, however, identify situations that may limit the effectiveness of by-product management:

1. *Non-operational treatment installations*. As indicated in Tukker 2004, vent-gas incinerators are assessed to be out of service for a about 1 to 2 percent of the time. If this down-time occurs during normal production and no back-up system is available²³, direct emissions of untreated streams are possible. These situations are strictly regulated in permits. Although such situations occur only during a limited amount of time, the contribution of untreated emissions can still contribute significantly, relative to the emissions during normal operation;
2. *Treatment techniques that may not remove unidentified and unanticipated substances*. Treatment techniques are designed to remove specific (identified) substances. There is therefore no guarantee that the treatment step is effective for previously unidentified components;

²³ The situation for MSWI installations is different, due to the use of parallel incinerators/flue-gas treatment. In case of malfunction of a flue-gas treatment installation, the air stream is diverted to the other treatment installation. Some, but not all, industrial plants have similar back-up systems.

3. *Operational mistakes.* Although obviously not intended, and although safety/back-up installations or measures are present in many cases, the possibility of faulty processing of waste streams due to human error cannot be fully excluded. Despite all efforts, situations can occur in which a certain risk of emissions still remains.

Based on these considerations, we conclude that emissions due to the limitations in by-product management cannot be fully excluded *anno* 2004.

7. Conclusions

General conclusions

- The previous chapters have arrived at the conclusion that substances with (potential) PBT properties are present in varying (small) amounts and types in chlorine-chain processes. Apparently because of improvements in production processes and the effective management of waste, atmospheric emissions and effluents (as described in Section 6.3), however, the current emissions are expected to be minor or at least limited. Possible PBTs were observed in various products, as were PCDD/Fs. Emissions may occur as a result of product use, atmospheric emissions or a limited number of effluents. Compared to current, known emissions with PBT character or emissions of dioxins from other sources (using worst-case estimates), these additional emissions from the chlorine chain are not expected to increase exposure levels on a national scale significantly;
- Emissions and effects were not distributed evenly over the different production processes, sites or products of the chlorine chain, but were confined to a limited number of production sites and products. These results indicate that there are no general problems concerning chlorinated organic microcontaminants with PBT properties that affect the entire chlorine chain; only a limited number of possible problem-cases were observed in this study.

Specific conclusions

Effluents

- Toxicological and biological testing revealed that none of the extracts of the aquatic effluents was responsive, either in the *in vivo* zebra fish ELS test or in the *in vitro* Carp-hep tests for cytotoxicity or estrogenicity. A varying number of industrial effluents were responsive in one or more of the other tests (Microtox *in vivo* test, Carp-hep EROD and DR-CALUX *in vitro* assays), as was the cooling water sample;
- Of the sixteen chlorine chain effluents tested, eleven yielded a response in the DR-CALUX test without acidic clean-up (with fairly high uncertainty about the presence of dioxins and/or dioxin-like compounds). The DR-CALUX load based on total extract was large enough to give a potential significant contribution to the chlorine chain total (see Figure 5.5) for only four of these effluents, in addition to the cooling water sample. For the load based on refined fractionation and after acidic clean-up (less uncertainty about the presence of dioxins and/or dioxin-like compounds) only one industrial effluent and the cooling water sample gave a relevant contribution.

Vent gases

- Dioxins (PCDD/Fs) were found in all of the flue gases from all three of the vent-gas installations and from the MSWI;
- All samples were responsive in the DR-CALUX test (after acidic clean-up). Contributions from analytically confirmed PCDD/Fs explained most (possibly as much as 80 to 90%) of the response after acidic clean-up for three of the four emissions. Only 22% of the response in one vent gas installation was attributable to dioxins;

- A few other PBTs (e.g., hexachlorobenzene, pentachlorobenzene), and two unidentified chlorinated compounds were found in the flue gases of one or more installations. The load of dioxins and/or dioxin-like compounds associated with the three vent-gas emissions is insignificant, relative to the level of known dioxin emissions in the Netherlands.

Products

- Of the eleven products that were tested, five were responsive; only three gave quantifiable responses in the DR-CALUX test after acidic clean-up (Figure 5.4): trichloroethylene (TRI), hydrochloric acid (HCl) and chloroprene rubber (CR). Additional chemical analysis confirmed the presence of dioxins (PCDD/Fs) in these samples (0.3-90 pg/g WHO-TEQ), but they could explain only 5 to 21% of the observed DR-CALUX response;
- The annual load attributed to chloroprene rubber (1.7 g bio-TEQ/y) by far dominates the total annual DR-CALUX load of chlorine-chain products (Table 5.6);
- The estimated loads of chlorinated impurities in six products may reach the kg/y level: chloroprene rubber (CR), monochloro-acetic acid (MCA), 1,2-dichloroethane (EDC), allylchloride (AC), trichloroethylene (TRI), and hydrochloric acid (HCl) (Table 5.5);
- Although some of the chlorinated compounds with PBT properties identified in products may be considered harmful to the environment, preliminary estimates of the total quantities emitted on a national scale are low, in comparison to known emissions of chlorinated substances that OSPAR classifies as hazardous. It is not likely that this will significantly increase the level of environmental exposure to these compounds on a national scale. Some of the impurities that are present in some of the products may not be emitted to the environment, as these products are intermediates used in subsequent production processes.

Unanticipated results

Another conclusion that can be drawn is that, despite the apparent effectiveness of by-product management, a number of unanticipated results occurred in the analyses of products; these results may constitute an area of concern that calls for further attention in risk-assessment frameworks.

- Chloroprene rubber (CR) was not considered a priority product within the priority-setting procedure adopted in this study (Section 2.2), but was added to the list as a 'wild card' product proposed by environmental NGOs. This product turned out to be the most responsive product in the DR-CALUX assay; it also contained the highest levels of chlorinated impurities (Table 5.5);
- Prior to the OVOC study, the supplier of the MCA sample was unaware of the presence of chlorinated alkylbenzenes; these impurities are very unlikely based on the raw materials used. From regular routine measurements by the supplier, only chlorinated aliphatic compounds were known as impurities. Similarly, the detection of chlorinated benzenes, -phenols, and -xylenes in HCl was an unanticipated result from the present study;
- A range of substances, both linear and cyclic, was found in the trichloro-ethylene sample. These substances were probably produced by a condensation reaction, be-

- ginning with trichloroethylene itself as precursor. The literature contained no previous reports of these substances as impurities in trichloroethylene;
- Dioxins (PCDD/Fs) were observed in HCl, TRI and CR (in concentrations corresponding to 0.3, 0.7 and 90 pg/g WHO-TEQ, respectively). This was a result that was not previously known from literature sources.

Uncertainties

- The study was partially exploratory. To the greatest extent possible, the methodology was based on validated methods (e.g., zebra fish ELS test, HRGCMS analysis of dioxins). Such methods were not always available for all of the sample types that were included in the study. When less validated methods were applied (e.g., *in vitro* testing of effluents, semi-chronic Microtox test, automated GC-MS identification studies) we took care to discuss them – and any possible limitations and uncertainties associated with them – with a wide range of scientists from both academia and industry. In some cases, additional validation studies were executed (acidic clean-up, fractionation) within the current study;
- It is impossible to quantify the uncertainties involved in the final overall results. Neither the qualitative analysis of the methodological limitations and uncertainties involved in estimating annual loads (Section 6.2), however, nor the application of worst-case approximations provide any indication that a more detailed and more comprehensive sampling design would have led to fundamentally different overall conclusions.

Part 4 References and Appendixes

8. References

Technical Reports from the OVOC project

- Belfroid, A.C., Stroomberg, G.J., Letcher, R.J., Stam, G.J. & van Hattum, B. (2000). *Chemical and bioscreening in search of toxic chlorine compounds in waste water. Integrative report on the results of the pilot study OVOC*. IVM Report (W-00/10), Institute for Environmental Studies, Vrije Universiteit, Amsterdam.
- De Koning, A. & Tukker, A. (2004). *Literature study on the importance of current anthropogenic PCDD/PCDF emissions in the Netherlands*. CML-IE Working Paper 2004.001. Centre of Environmental Science, Leiden University, Leiden.
- Gerritsen, A. (2004). *Comparison of ecotoxicity of effluents from chlorine chain industry with effluents from other industrial sectors*. RIZA notanr. 2004.078. RIZA, Lelystad.
- Kleijn, R., Elshkaki, A., de Koning, A. & Tukker, A. (2001a). *Literature study on unknown organochlorine compounds in the environment from anthropogenic sources*. CML-SSP Working Paper 2001.001. Centre of Environmental Science, Leiden University, Leiden.
- Kleijn, R., Elshkaki, A., de Koning, A. & Tukker, A. (2001b). *Literature study on degradation products of known emissions*. CML-SSP Working Paper 2001.001. Centre of Environmental Science, Leiden University, Leiden.
- Lamoree, M.H., Stroomberg, G.J., Besselink, H., Senhorst, H., Sanderson, T. & van Hattum, B. (2003). *Chlorinated Micropollutants in Aquatic Effluents. Part-1 Part-1 Sampling, pre-treatment, classification and primary effect screening*. IVM Report (E-03/02). Institute for Environmental Studies, Vrije Universiteit, Amsterdam.
- Lamoree, M.H., Sanderson, T., Senhorst, H., Leonards, P. & van Hattum, B. (2004a). *Chlorinated Micropollutants in Aquatic Effluents. Part-3 Refined fractionation and primary TIE studies*. IVM Report (E-04/01). Institute for Environmental Studies, Vrije Universiteit, Amsterdam.
- Lamoree, M., Swart, K., Senhorst, H. & van Hattum, B. (2004b). *Validation of the acidic sample clean-up procedure for the DR-CALUX assay*. IVM Report (L-04/02). Institute for Environmental Studies, Vrije Universiteit, Amsterdam.
- Senhorst, H., Lamoree, M.H., van der Linden, S., Sanderson, T. & van Hattum, B. (2004a). *Chlorinated Micropollutants in Aquatic Effluents. Part-2 Biodegradation studies on selected effluents (Workpackage 8)*. IVM Report (E-04/02). Institute for Environmental Studies, Vrije Universiteit, Amsterdam.
- Senhorst, H., Lamoree, M.H., van der Linde, S., Sanderson, T., van Stee, L.L.P. & van Hattum, B. (2004b). *Chlorinated Micropollutants in Aquatic Effluents. Part-4 In-pant TIE studies (Workpackage 9)*. IVM Report (E-04/03). Institute for Environmental Studies, Vrije Universiteit, Amsterdam.
- Tukker, A. (2000a). *Chlorinated micropollutants: priority setting. Report on Task 3.1 and 3.2 of the preliminary investigation of the chlorine micropollutant study*. Report nr. STB-00-34, TNO-STB, Delft.
- Tukker, A. (2000b). *Chlorinated micropollutants: Literature search on emissions. Backgrounds to the priority setting for the main research phase*. Reportnr. STB-00-35. TNO-STB, Delft.
- Tukker, A. (2004). *Measures taken on 3 Dutch industrial sites to prevent emissions of chlorinated (micro)emissions between 1985 and 2002*. Report nr. STB-04-03. TNO-STB, Delft.

- Van Hattum, B., Swart, K., Lamoree, M.H., van der Linden, S., Senhorst, H., de Voogt, P. & Tukker, A. (2004b). *Chlorinated Micropollutants in Products*. IVM Report (E-04/05). Institute for Environmental Studies, Vrije Universiteit, Amsterdam.
- Van Hattum, B., Peters, R., Timmer, J., Swart, K., Lamoree, M.H., van der Linden, S., Leonards, P. & Felzel, E. (2004a). *Chlorinated Micropollutants in Atmospheric emissions*. IVM Report (E-04/04). Institute for Environmental Studies, Vrije Universiteit, Amsterdam.

Other references cited in the text

- Aarts, J.M.M.J.G. & Palmer, R. (2002). Established and deduced aromatic hydrocarbon (Ah) receptor (AhR) agonists and their relative potency. Wageningen University, Department of Agrotechnology and Food Sciences, Wageningen.
- Aerts J. & Denison M *et al.* (1995). Species-species antagonism of Ah-receptor action by 2,2',5,5'-tetrachloro- and 2,2',3,3',4,4'-hexachlorobiphenyl. *Eur J Pharm Environ Toxicol Sect*, 293, 463-474.
- Ahlborg, U.G., Becking, G.C., Birnbaum, L.S., Brouwer, A., Derks, H.J.G.M., Feeley, M., Golor, G., Hanberg, A., Larsen, J.C., Liem, A.K.D., Safe, S.H., Schlatter, C., Waern, F., Younes M. & Yrjan-heikki, E. (1994). Toxic Equivalency Factors for Dioxin Like PCBs Report on a WHO ECEH and IPCS Consultation. December 1993. *Chemosphere* 28, 1049-1067.
- Barreveld, H.L., Berbee, R.P.M. & Ferdinandy, M.M.A. (2001). *Vergeten stoffen in Nederlands oppervlaktewater*. RIZA rapport nr. 2001.020. RIZA, Lelystad (in Dutch).
- Behnisch, P.A., Hosoe, K. & Sakai, S.i. (2001). Bioanalytical screening methods for dioxins and dioxin-like compounds -- a review of bioassay/biomarker technology. *Environment International*, 27, 413-439.
- Behnisch, P.A., Hosoe, K. & Sakai, S.i. (2003). Brominated dioxin-like compounds: in vitro assessment in comparison to classical dioxin-like compounds and other polyaromatic compounds. *Environment International*, 29, 861-877.
- Benisch P *et al.* (2002). Screening of Dioxin-Like Toxicity Equivalents for Various Matrices with Wildtype and Recombinant Rat Hepatoma H4IIE Cells. *Toxicological sciences*, 69, 125-130.
- Berends, A.G., Boelhouwers, E.J., Thus, J.L.G., De Gerlache, J. & De Rooij, C.G. (1997). Bioaccumulation and lack of toxicity of octachlorodibenzofuran (OCDF) and octachlorodibenzo-p-dioxin (OCDD) to early-life stages of zebra fish (*Brachydanio rerio*). *Chemosphere*, 35, 853-865.
- Bremmer, H.J, Troost, L.M., Kuipers, G., De Koning, J. & Sein, A.A. (1993) *Emissies van dioxinen in Nederland*. RIVM, TNO Rapport 770501003. TNO, Apeldoorn (in Dutch).
- Bremmer, H.J. (1991) *Bronnen van dioxines in Nederland*. RIVM-rapport nr 730501014. RIVM, Bilthoven (in Dutch).
- Carey, J. (ed) (1998). *Ecotoxicological Risk Assessment of the Chlorinated Organic Chemicals*, SETAC Press ISBN 1880611074.
- De Leer, E.W.B. (1985). The identification of highly chlorinated ethers and diethers in river sediment near an epichlorohydrin plant. *Water Res.* 19, 1411-1419.
- Dugan, S. (2000). Comparison of gas chromatography-pulsed flame photometric detection-mass spectrometry, automated mass spectral deconvolution and identification system and gas chromatography-tandem mass spectrometry as tools for trace level detection and identification. *Journal of Chromatography A*, 868, 229-247.
- EC (2001). Council regulation (EC) No 2375/2001 of 29 November 2001 amending Commission Regulation (EC) No 466/2001 setting maximum levels for certain contaminants in foodstuffs. *EN Official Journal of the European Communities* 6.12.2001 L 321/1-5.

- Elonen, G.E., Spehar, R.L., Holcombe, G.W., Johnson, R.D., Fernandez, J.D., Tietge, J.E. & Cook, P.M. (1998). *Toxicity of 2,3,7,8-TCDD to seven freshwater fish species*. US-Environmental Protection Agency, Mid-Continent Ecology Division, Duluth, USA.
- EMEP (2003). Transboundary pollution by HM and POPs; Netherlands. MSC-E Note 6/2003. August, 2003. Meteorological synthesizing Centre-East, Moscow, Russia.
- Emissiemonitor (2000). Jaarcijfers 2000 en ramingen 2001 voor emissies en afval. Emissieregistratie, TNO-MEP, Apeldoorn (in Dutch).
- Emissiemonitor (2002). Rapportagereeks Milieumonitor nr. 6, november 2002. Emissieregistratie, TNO-MEP, Apeldoorn (in Dutch).
- Garrison, P. M., Tullis, K., Aarts, J. M., Brouwer, A., Giesy, J. P. & Denison, M. S. (1996). Species-specific recombinant cell lines as bioassay systems for the detection of 2,3,7,8-tetrachlorodibenzo-p-dioxin-like chemicals. *Fundam Appl Toxicol*, 30, 194-203.
- Giesy, J.P., Ludwig, J.P. & Tillitt, D.E. 1994b. Deformities in birds of the Great Lakes region: assigning causality. *Environ. Sci. Toxicol.*, 28, 128A-135A.
- Giesy, J.P., Hilscherova, K., Jones, P.D., Kannan, K. & Machala, M. (2002). Cell bioassays for detection of aryl hydrocarbon (AhR) and estrogen receptor (ER) mediated activity in environmental samples. *Marine Pollution Bulletin*, 45, 3-16.
- Hilscherova, K., Machala, M., Kannan, K., Blankenship, A.L. & Giesy, J.P. (2000). Bioassays for detection of aryl hydrocarbon (AhR) and estrogen receptor (ER) mediated activity in environmental samples. *Environ. Sci. Pollut. Res.*, 7, 159-171.
- Houtman, C.J., Cenijn, P.H, Hamers, T., Lamoree, M.H., Legler, J., Murk, A.J. & Brouwer, A. (2004) Toxicological profiling of sediments using in vitro bioassays, with emphasis on endo-crine disruption. *Environmental Toxicology and Chemistry*, 23, 32-40.
- Leonards, P.E.G. & van der Veen. I. (2001). *Chemische validatie van analysemethoden in sediment voor de toepassing van de DR-Calux*. RIVO rapport no. C076/01, RIVO, IJmuiden (in Dutch).
- Machala, M., Vondracek, J., Blaha, L., Ciganek, M. & Neca, J.V. (2001). Aryl hydrocarbon receptor-mediated activity of mutagenic polycyclic aromatic hydrocarbons determined using in vitro reporter gene assay. *Mutat Res*, 497, 49-62.
- Mallard, W.G. & J. Reed (1997). *Automated Mass Spectral Deconvolution & Identification System AMDIS — user guide*. U.S. Department of Commerce Technology Administration, National Institute of Standards and Technology (NIST) Gaithersburg, MD 20899, USA.
- Matsui, M., Kashima, Y., Kawano, M., Matsuda, M., Ambe, K., Wakimoto, T. & Doi, R. (2003). Dioxin-like potencies and extractable organohalogen (EOX) in medical, municipal and domestic waste incinerator ashes in Japan. *Chemosphere*, 53, 971-980.
- McLafferty F.W., & Turecek F. (1993). In: McLafferty, F.W. Interpretation of mass spectra, 4th edition, 1993, Californina University Science Books, Mill Valley, USA. p. 283-291.
- Meylan, W. & Howard, P. (1999b). *User's guide for HENRYWINc Version 3, Henry's law constant pro-gram for Microsoft Windows*. Syracuse Research Corporation, North Syracuse (NY).
- Meylan, W. & Howard, P. (1999c). *User's guide for KOWWINc Version 1.6, Log Octanol-Water Partition Coefficient Estimation Program for Microsoft Windows*. Syracuse Research Corporation, North Syracuse (NY).
- Murk, A.J., Legler, J., Denison, M.S., Giesy, J.P., van de Guchte, C. & Brouwer, A. (1996). Chemical-activated luciferase gene expression (CALUX): a novel in vitro bioassay for Ah receptor active compounds in sediments and pore water. *Fundam Appl Toxicol.*, 33, pp. 149-160.

- Murk, A.J., Leonards, P.E.G., van Hattum, B., Luit, R., van der Weiden, M.E.J. & Smit, M. (1998). Application of biomarkers for exposure and effect of polyhalogenated aromatic hydrocarbons in naturally exposed European otters (*Lutra lutra*). *Environ. Toxicol. Pharm.*, 6 (2), 91-102.
- Murk, A.J., Denison, M.S., Leonards, P.E.G., Bulder, A.S., Jonas, A.S., Rozemeijer, M.J.C., Koeman, J.H. & Brouwer, A. (1997). The CALUX assay adapted and validated for measuring TCDD equivalents in blood plasma. *Environ. Toxicol. Chem.*, 16, pp.1583-1589.
- Murk et al. (1998). *Application of biomarkers for exposure and effect of polyhalogenated aromatic hydrocarbons in naturally exposed European otters (Lutra lutra)*.
- NATO/CCMS (1988). *Scientific basis for the development of the International Toxicity Equivalency Factor (I-TEF) method of risk assessment for complex mixtures of dioxins and related compounds*. North Atlantic Treaty Organisation, Brussels. OSPAR (1988). OSPAR Strategy with regard to Hazardous Substances. Appendix-3. List of Candidate Substances.
- NEN (1997a). NEN-EN 1948-1: *Emissies van stationaire bronnen. Bepaling van de concentratie aan PCDD's/PCDF's*. Deel 1: Monsterneming. Januari 1997. NEN, Delft (in Dutch).
- OSPAR (1998). *OSPAR Strategy with regard to Hazardous Substances*. Appendix-3. List of Candidate Substances.
- OSPAR (2002). *The prevention of pollution by hazardous substances*, OSPAR, London, January 2002.
- Pacyna, J.M., Breivik, K., Münch, J. & Fudala, J. (2003). European atmospheric emissions of selected persistent organic pollutants, 1970-1995. *Atmospheric Environment* 37, S119-S131.
- Pluim, H.J., Slot, P.C., Olie, K., van der Slikke, J.W. & Kopper, J.G. (1992). Diurnal variations in concentrations of PCDDs and PCDFs in human milk. *Chemosphere*, 25, 307-311.
- Quaß, U., Fermann, M. & Bröker, G. (2000). *The European Dioxin Emission Inventory Stage II Volume 3 Assessment of dioxin emissions until 2005*. Report produced by the North Rhine Westphalia State Environment Agency on behalf of the European Commission DG Environment.
- Quaß, U., Fermann, M. & Bröker, G. (2004). The European dioxin air emission inventory project - final results. *Chemosphere*, 54, 1319-1327.
- RIVM (1999). *Milieubalans 99*, Samson H. D. Tjeenk Willink bv, Alphen aan den Rijn (in Dutch).
- RIVM (2002). *Milieubalans 2002; Het Nederlandse milieu verklaard*. Kluwer Alphen aan den Rijn (in Dutch).
- RIZA (1995). *Beoordeling van milieubezwaarlijkheid van chloorethers ontstaan bij de productie van epichloorhydrine (ECH)*. RIZA report 94.047. RIZA, Lelystad (in Dutch).
- Safe, S.H. (1990). Polychlorinated biphenyls (PCBs), dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs) and related compounds: environmental and mechanistic considerations which support the development of toxic equivalency factors (TEFs). *CRC Crit. Rev. Toxicol.*, 21, 51-71.
- Sanderson, J.T., Aarts, J.M., Brouwer, A., Froese, K.L., Denison, M.S. & Giesy, J.P. (1996). Comparison of Ah receptor-mediated luciferase and ethoxyresorufin-O-deethylase induction in H4IIE cells: implications for their use as bioanalytical tools for the detection of polyhalogenated aromatic hydrocarbons. *Toxicol Appl Pharma-col*, 137, 316-325.
- Sanderson, J.T., Elliott, J.E., Norstrom, R.J., Whitehead, P.E., Hart, L.E., Cheng, K.M. & Bellward, G.D. (1994a). Monitoring biological effects of polychlorinated dibenzo-p-dioxins, dibenzofurans and biphenyls in great blue heron chicks (*Ardea herodias*) in British Columbia. *J. Toxicol. Environ. Health.*, 41, 435-450.

- Sanderson, J.T., Norstrom, R.J., Elliott, J.E., Hart, L.E., Cheng, K.M. & Bellward, G.D. (1994b). Biological effects of polychlorinated dibenzo-p-dioxins, dibenzofurans and biphenyls in double-crested cormorant chicks (*Phalacrocorax auritus*). *J. Toxicol. Environ. Health*, *41*, 245-263.
- Santos, F.J. & Galceran, M.T. (2002). The application of gas chromatograph to environmental analysis. *Trends in Anal. Chem.*, *21*, 672-684.
- Schoeters, G., Goyvaerts, M.P., Ooms, D. & Van Cleuvenbergen, R. (2004). The evaluation of dioxin and dioxin-like contaminants in selected food samples obtained from the Belgian market: comparison of TEQ measurements obtained through the CALUX bioassay with congener specific chemical analyses. *Chemosphere*, *54*, 1289-1297.
- Schramm, K.A., Klimm C. a, Hofmaier A. b & Kettrup, A. (2001). Comparison of dioxin-like-response in vitro and chemical analysis of emissions and materials. *Chemosphere* *42*, 551-557.
- Schrenk, D., Lipp, H. & Wiesmuller, P. etc. (1991). Assessment of biological activities of mixtures of polychlorinated dibenzo-p-dioxins: comparison between defined mixtures and their constituents. *Arch. Toxicol*, *65*, 114-118.
- Smeets, J. et al. (1999). The anti-estrogenicity of Ah receptor agonists in carp (*Cyprinus carpio*) hepa-tocytes. *Toxicol. Sci.*, *52*, 178-88.
- Stronkhorst, J., Leonards, P. & Murk, A.J. (2002). Using the dioxin receptor-calux in vitro bioassay to screen marine harbor sediments for compounds with a dioxin-like mode of action. *Environmental Toxicology and Chemistry*, *21*, 2552-2561.
- Tillitt, D.E., Ankley, G.A., Giesy, J.P., Ludwig, J.P., Kurita-Matsuba, H., Weseloh, D.V., Ross, P.S., Bishop, C., Sileo, L., Stromberg, K.L., Larson, J. & Kubiak, T.J. (1992). Polychlorinated biphenyl residues and egg mortality in double-crested cormorants from the Great Lakes. *Environ. Toxicol. Chem.*, *11*, 1281-1288.
- Tukker A, Kleijn, R. & v.d. Voet, E. (1995). *A chlorine balance for the Netherlands*. TNO-STB and CML, TNO-STB, Apeldoorn.
- Tukker, A., Kleijn, R. & Smeets, W.R.W. (1997). Chlorine in the Netherlands, part III – Risk management in uncertainty for chlorine. *J. Industrial Ecology*, *1*, pp.91-110.
- UNEP (2001). *Standardized Toolkit for Identification and Quantification of Dioxin and Furan Releases*. Draft. UNEP Chemicals Switzerland.
- Van den Berg, M., Birnbaum, L., Bosveld, B. T. C., Brunstrom, B., Cook, P., Feely, M. & Giesy, J. P. (1998). Toxic equivalency factors (TEFs) for PCBs, PCDDs and PCDFs for humans and wildlife. *Environ. Health Perspect.*, *106*, 775-792.
- Van Overmeire, I., Clark, G.C., Brown, D.J., Chu, M.D., Marcus Cooke, W., Denison, M.S., Baeyens, W., Srebrnik, S. & Goeyens, L. (2001). Trace contamination with dioxin-like chemicals: evaluation of bioassay-based TEQ determination for hazard assessment and regulatory responses. *Environmental Science & Policy*, *4*, 345-357.
- Van Zorge, J.A., van Wijnen, J.H., Theelen, R.M.C., Olie, K. & van den Berg, M. (1989). Assessment of the toxicity of mixtures of halogenated dibenzo-p-dioxins and dibenzofurans by use of toxicity equivalence factors (TEF). *Chemosphere*, *19*, 1881-1895.
- Villeneuve, D.L., Khim, J.S., Kannan, K. & Giesy, J.P. (2001). In vitro response of fish and mammalian cells to complex mixtures of polychlorinated naphthalenes, polychlorinated biphenyls, and polycyclic aromatic hydrocarbons. *Aquatic Toxicology*, *54*, 125-141.
- Walker, M.K., Spitsbergen, J.M., Olson, J.R. & Peterson, R.E. (1991). 2,3,7,8-TCDD toxicity during early life stage development of lake trout (*Salvelinus namaycush*). *Can. J. Fish. Aquat. Sci.* *48*, 875-883.

9. Appendixes

List of Appendixes

- 9.1 Priority setting procedure for sampling programme
- 9.2 Studies facilitating the interpretation of results
- 9.3 Publications and reports from OVOC project
- 9.4 Summary information on DR-CALUX and Carp-Hep assays
- 9.5 PBT definitions in regulatory frameworks
- 9.6 Analysis of GC-MS data and identification with AMDIS-NIST
- 9.7 In-plant TIE study
- 9.8 Biodegradation study
- 9.9 Extensive analysis of DR-CALUX response in MCD samples
- 9.10 Glossary (abbreviations)
- 9.11 BOVOC steering committee participants
- 9.12 List of Tables and Figures

9.1 Priority-setting procedure for sampling programme

9.1.1 Processes and products in the Dutch chlorine chain

Figure 2.1 reviews the chlorine chain in the Netherlands in 1990. In view of the formation of PBTs, it is possible to distinguish various types of processes:

1. Processes in the chlorine industry, where chlorine is used as a building block in chemical reactions. In this case, unwanted by-products may arise that could be emitted either to air or to water. The number of such plants in a country like the Netherlands is rather limited (several dozen). For such processes, *point source emission measurements* are the logical approach to detect formation of yet unidentified chlorinated PBTs;
2. Processes, where chlorinated products are used (e.g., solvents) but not transformed into other compounds. This concerns literally thousands of production locations in a country like the Netherlands. In such processes, the chlorine is not available in active form. As a working hypothesis for this study, conversion of such compounds to other chlorinated PBTs is not likely. Yet, the products of course are proceeding from the processes listed under 1), and hence in principle, if formation of by-products takes place there, they may be present in the product²⁴. Experience shows that such contaminants may be released at other locations. For this group, *testing products on yet unidentified by-products* is probably the most effective way to determine whether problems in the downstream chain can be expected;
3. Processes in which active chlorine is used as a means of preventing algae and bacteria growth (e.g., hypochlorite)²⁵. In this case, the chlorine is available in active form, and the formation of by-products like trihalomethanes is a well-known phenomenon. As in the first point, *point source emission measurement* is also the logical approach to detecting the formation of yet-unidentified chlorinated PBTs in this case as well;
4. Processes in which chlorine plays no main role, but is present in inorganic or other forms and may thus be converted into more harmful compounds. The main examples in this case are municipal solid waste incinerators (MSWIs) and vent-gas incineration systems (c.f., Bremmer et al., 1994). In this case as well, *point source emission measurement* is the logical approach for detecting the formation of yet-unidentified PBTs.

²⁴ Only rarely is a product from the chemical industry produced as a 100.00 % pure grade; in practice, minor amounts of by-products may be present. Even though this generally poses no problem for the subsequent use of these products, the possibility that such impurities may form a source of pollution downstream in the production chain cannot be excluded. Mobius (1990) provided a good example of this. It appeared that a paper industry that used no chlorinated products emitted significant amounts of AOX. On further investigation, the industry had apparently used a *chlorine-free* paper improver that had been produced with ECH (a chlorinated compound). Impurities in the ECH appeared to be present in the paper improver.

²⁵ Another important application is paper and pulp bleaching; this is virtually absent in the Netherlands.

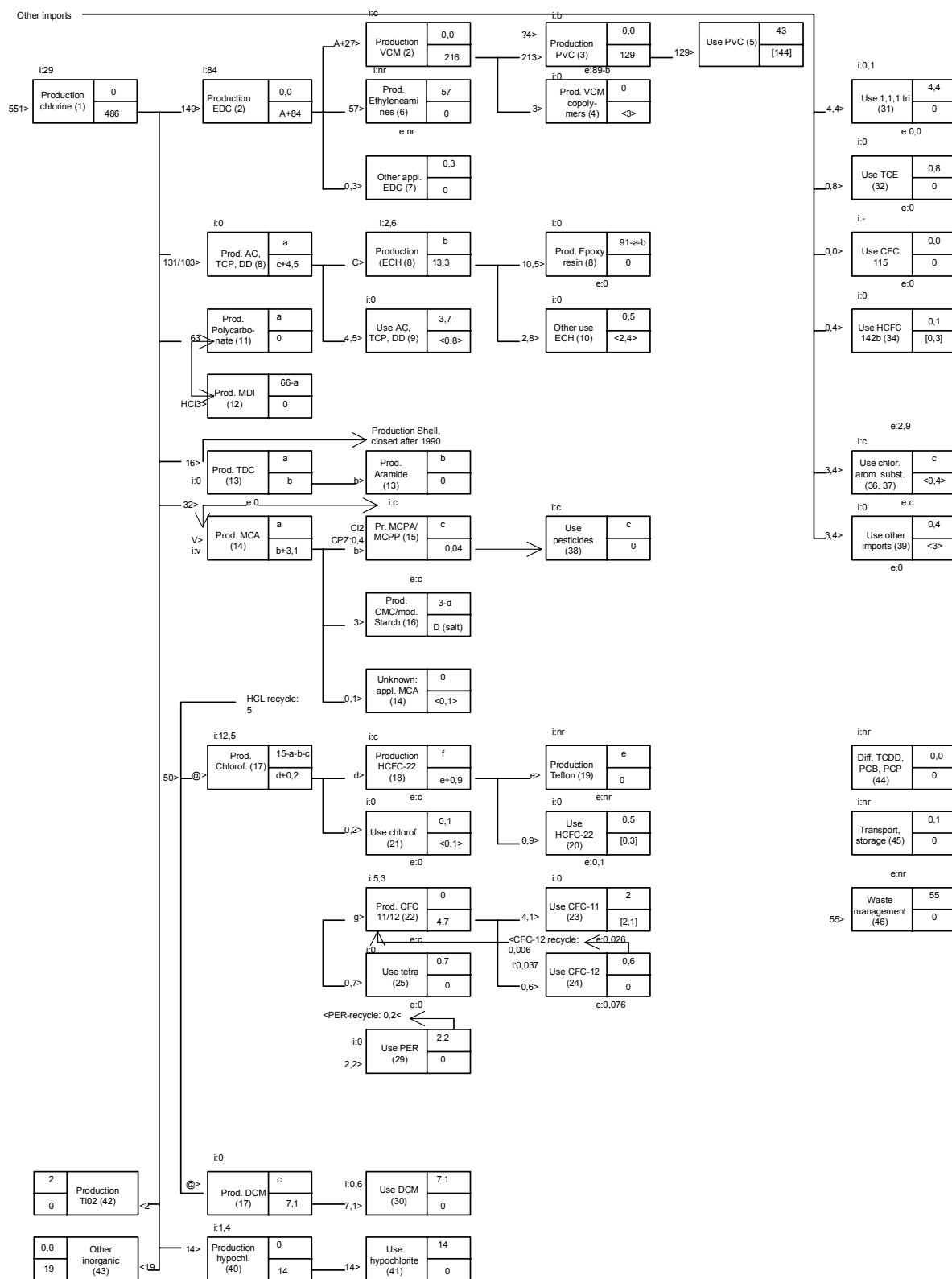


Figure 9.1.1 The Dutch chlorine chain in 1990 (values in ktpa). For legends and explanation, see next page.

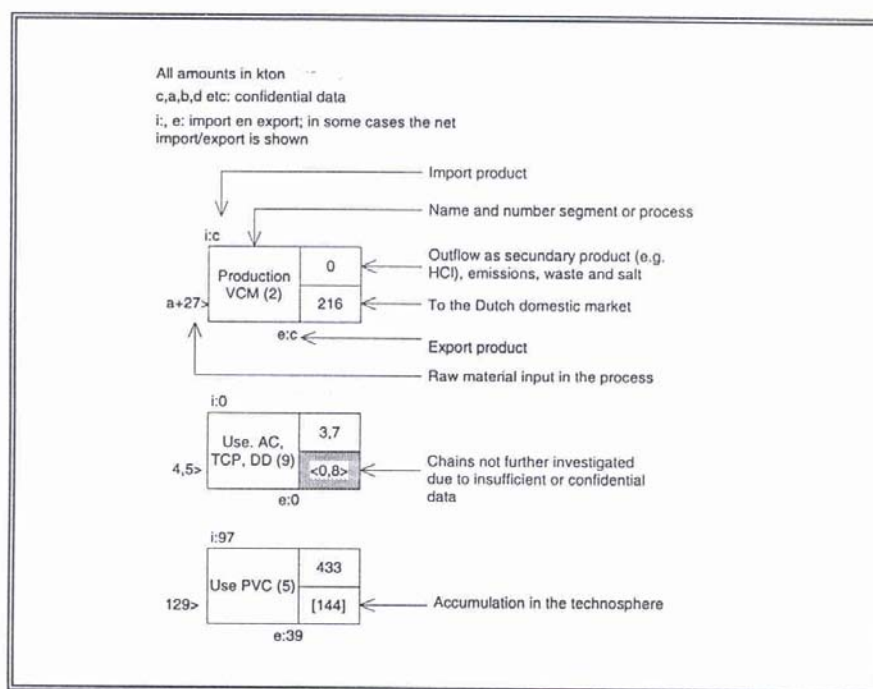


Figure 9.1.2 Legend for substance-flow diagram (Figure 9.1.1).

9.1.2 Criteria for priority setting

In the next sections, we will discuss priority setting for each category we discerned in Chapter 2.2.1. In this priority setting, a number of common elements can be used to rank processes/products according to the chance that they may contribute significantly to a chlorinated micropollutant problem:

1. The chlorine throughput in a process, or the volume of product use. In such cases, even small percentages of unknown emissions and unknown contamination may lead to significant micropollutant problems;
2. The volume of known chlorinated emissions. Also here, even small percentages of by-products and/or unknown emissions may lead to a significant micropollutant problem;
3. The chance of formation of by-products. In general, the literature mentions the following criteria (see e.g., the dioxin reviews by Bremmer, 1994 and Fiedler, 1994):
 - The presence of active chlorine;
 - The availability of precursors that, together with active chlorine, may easily form 'complex' compounds²⁶;
 - The presence of a catalyst or UV-light;

²⁶ For instance, if organic components with ring structures are already available in raw materials, there is a higher chance for the formation of aromatic chlorines. For instance, Fiedler (1994) claims that the chance of finding dioxins as a by-product in the production of the following compounds decreases in the following order: chlorophenoles > chlorobenzenes > aliphatic organochlorines > inorganic chlorine compounds

Table 9.1.1 Production processes in the Dutch chlorine chain.

<ol style="list-style-type: none"> 1. The production of chlorine (Akzo Nobel Rotterdam, Delfzijl and Hengelo) 2. The production of EDC and VCM (Shin-Etsu, located on the site of Akzo Nobel Rotterdam); 3. The production of MCPA and MCPP (two pesticides; by Nufarm, located on the site of Akzo Nobel Rotterdam); 4. The production of allylchloride, epichlorohydrine and epoxyresins (at Shell Nederland Chemie, Rotterdam); 5. The production of PVC (from VCM; Shin-Etsu, located on the site of Shell Chemie Nederland Rotterdam and Tessenderloo chemie, located on the site of DSM in Geleen); 6. The production of PVC copolymers (at BASF, Arnhem and Vinamul, Geleen); 7. The production of monochloroacetic acid (MCA) (at Akzo Nobel Hengelo) 8. The production of chlorinated solvents (DCM, chloroform; Akzo Nobel Delfzijl); 9. The production of TDC and aramide (Aramid Products, part of Acordis, Delfzijl); 10. The production of ethyleneamines (at Dow Benelux B.V., Terneuzen, and Delamine, a joint venture of Akzo Nobel and Tosoh, Delfzijl); 11. Production of C-PVC, BF Goodrich, Delfzijl; 12. The production of polycarbonate (General Electric Plastics, Bergen op Zoom); 13. The production of MDI (Huntsman ICI, Botlek); 14. The production of HCFC-22 and Teflon (Du Pont de Nemours, Dordrecht); 15. The production of CFC 11 and 12 for essential applications still allowed under the Montreal Protocol c.a. (Allied Signal, Weert); 	<ol style="list-style-type: none"> 16. The production of products from MCA <ul style="list-style-type: none"> • CMC (Metsa Serla, Nijmegen and Akzo Nobel, Arnhem); • Modified starch (Avebe, Foxhol and de Bijenkorf, Koog aan de Zaan); 17. Production of chemicals using aromatic chlorine compounds <ul style="list-style-type: none"> • Production of pesticides (Uniroyal, Amsterdam); • Production of organotin (Elf Atochem, Terneuzen); • Production of benzylalcohol (Tessenderloo chemie located on the site of Akzo Nobel, v/h Eka Nobel, Maastricht); • Production of pharmaceuticals (DSM Andeno Maarssen and Solvay Pharmaceuticals, Weesp); 18. Production of ECH-derivates (e.g., modifiers of cellulose and starch, by Condea Servo Delden, Cytec Botlek, Hercules and Inspec Fine Chemicals (IFC); 19. Kemira Pigments (production of titane dioxide); 20. The production of pharmaceuticals, odorants, colorants and conservation agents with use of EDC (probably mainly as solvent) at DSM Fine Chemicals Venlo and Givaudan Rourde. 21. Several tank storage and/or transport firms (e.g., Paktank, C&B Botlek)*; 22. Several physical chemical waste treatment firms (e.g., ATM)*; 23. Several firms using chlorinated solvents*; 24. Kappa Recycled paper*; 25. Sappi (paper recycling)*; 26. Du Pont de Nemours Botlek (nylon production)*; 27. Shell Nederland Chemie Moerdijk*; 28. Brunner Mond (soda production)*; 29. Ciba Geigy Maastricht (production of specialities)*.
----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------	-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------

* These companies were not known to have plants using active chlorine, but showed up as emitters of AOX in WVO-Info, and were therefore included in the long list for priority setting.

- High temperatures;
- (For water emissions) A clear mismatch between the volume of known emitted components and the total EOX or AOX output;
- Indications from previous investigations or from the literature that by-products are formed.

Table 9.1.2 Products in the Dutch chlorine chain (produced and/or imported).

1. PVC;	12. Polycarbonate (ditto);
2. EDC in other applications than PVC production;	13. Pesticides (part produced inside and part produced outside the Netherlands);
3. ECH (in relation to the processes listed under Item 16 above);	14. HCl produced as a by-product in e.g., DCM production, EDC production and ECH production, and used off-site.
4. AC (in unknown applications);	15. PERC;
5. MCA (in relation to the processes listed under Item 18 above);	16. Trichloroethene;
6. DCM;	17. Chlorobenzenes;
7. Chloroform;	18. Chlorophenoles;
8. HCFCs;	19. Chloroethane;
9. Teflon;	20. Dichloropropane;
10. Ethyleneamines (in principle chlorine free, but a major product from the chlorine industry);	21. Chloroparaffins.
11. MDI (ditto);	

Table 9.1.3 Other processes (use of active chlorine as disinfectant and waste incineration).

1. Household applications;	4. Incineration of regular waste;
2. Swimming pools;	5. Incineration of hazardous waste.
3. Cooling water, including many industrial circuits.	

Although the latter criteria are mainly aimed at production processes, they can, of course, also be useful for ranking products. Products produced in processes with a high chance on the formation of by-products obviously have a higher chance of contamination than do those produced in other processes.

9.1.3 Results of the priority setting

A priority setting was performed in a number of steps. First, a quick inventory of the following elements was conducted for the remaining processes, products and other potential sources:

1. Production volumes or uses: taken from the Dutch chlorine-chain study;
2. Known emissions, particularly AOX to water: taken from national emission databases (ER-I and/or WVO-Info);
3. The probability of the formation of by-products: performed by determining the applicability of the criteria mentioned in Section 2.2.2, based on limited literature study with regard to the processes at stake.

The results of this exercise are shown in Table 9.1.4. Table 9.1.4 shows the same processes as given in Table 9.1.1 to 9.1.3, but organised in a somewhat different fashion, (i.e., by production location). In a rather pragmatic way, we combined the information in Table 9.1.4 in a number of ways:

- For cases in which the chlorine throughput was high, the organochlorine emissions were high and most of the indicators suggested that the chances of by-product formation were positive, the process was listed as a ‘hard’ priority;
- In some cases, research showed that some factories had closed or that some products had been (virtually) phased out. These were obviously no longer included as a priority. In those cases where the chance of by-products could almost be excluded (e.g., low chlorine flows, no AOX emissions, no factor contributing to a high chance on by-products), the process was listed as a ‘clear non-priority’;
- In some cases, two or more factories that were using essentially the same production processes were listed. In that case, one would be designated as a ‘non-priority’.

When we eliminated all ‘clear non-priorities’, it appeared that about 52 flows (emissions to air, emissions to water or product flows) would have to be analysed. This was still too many, given the budgetary constraints faced in the Main phase of the OVOC study. In discussion with the BOVOC Steering Committee, 12 potential sampling points were deleted from the list of 52. The rationale for this was:

- (For water emissions) A very low emission of EOX of 0 or 1 kg/annum (Solvay Duphar, Cytec, Ciba Geigy)²⁷, or an emission that was not monitored and that seemed to have no advantage for the Steering Committee (Condea Servo Delden and IFC);
- A large chance that emissions were related to solvent use (Du Pont Botlek, Shell Moerdijk);
- Probably no relation with the chlorine chain (soda production at Brunner Mond);
- (for air emissions) No clear indication that the plant at stake would have important emissions to air, in comparison to the other air emissions already sampled (Dow Terneuzen, BF Goodrich, Uniroyal);
- (For products) the least-used product (i.e., according to the Dutch chlorine-chain study: chloro-ethane).

²⁷ The regional water board suggested keeping Elf Atochem, Vlissingen in the sampling list, despite its low EOX emission.

In a further reduction process, the following sampling points were deleted:

- Products: HCFCs, as they will be phased out anyway after about 2010; ECH, as a number of processes using ECH were already covered and chloroform, as it is mainly used as a feedstock in other processes that are already covered;
- Air: DuPont de Nemours, Dordrecht and Akzo Nobel, Delzijl. This is a fully arbitrary choice, but the point with air emissions is that they all tend to occur through vent-gas incinerators. For the OVOC study, it suffices to check a few of such incinerators;
- Water: Akzo Nobel Maastricht. This process makes only minor use of chlorinated compounds and discharges into a sewage system. Furthermore, Avebe was deleted, as its emissions were likely to be related to the use of hypochlorite. The inclusion of Avebe would have added little value, as other uses of hypochlorite had already been covered.

The result of this selection process is given in Tables 2.1 and 2.2 (Chapter 2.1) This selection contains the final priorities that (in principle) had been checked in the practical research of the phase 2 of the OVOC study.

Table 9.1.4 Basic data for priority setting in the main research study on chlorinated micropollutants.

No	Firm name	Processes (between brackets: chapter in background document)	Use of chlorine compounds in ktpa (as given in chlorine-chain study or estimate)	Emission EOCL or AOX (order of magnitude, in kg)	Chance of by-products					
					Active chlor-ine	Precursors	Catalysts	High Temp.	Unexplained EOCL	Literature data
<i>Companies listed in chlorine-chain study</i>										
1	Akzo Nobel Botlek (VCM and MCPA/CMPP production owned by Shin-Etsu or Nufarm)	Chlorine production (2) EDC/VCM production (3) production of MCPA/MCPP (4)	<279.6 >149.2 <14.5	100	+	+	+	+	+	+
2	Shell Nederland Chemie (PVC Production owned by Shin-Etsu)	ECH and AC production (5) PVC Production (6)	131 213	2800	+	+	+	+	+	+
3	Limburgse Vinyl Maatschappij (at DSM)	PVC Production (6)	213	30						
4	BASF Nederland Arnhem	Production of PVC-copolymer (7)	3	not monitored						
5	Vinamul Geleen	Production of PVC-copolymer (7)	3	not monitored						
6	Akzo Nobel Hengelo	Chlorine production MCA production (8)	approx. 80 <14.5	not monitored	+			- , 85 C		
7	Akzo Nobel Farnsum/Delfzijl	Chlorine production Solvent production (DCM, chloroform) (9)	89 40	EOCL not monitored	+					+
8	Aramid Products, Delfzijl (part Acordis)	Production of aramide and Precursors (10)	<16	Chlorotoluene: 42	+	+	+	+		
9	Delamine, Delfzijl (joint-venture, Akzo Nobel)	Production of ethyleneamines (11)	57	not monitored				140 C		
10	Dow Benelux Terneuzen	Production of ethyleneamines (11)	57	76					+	

No	Firm name	Processes (between brackets: chapter in background document)	Use of chlorine compounds in ktpa (as given in chlorine-chain study or estimate)	Emission EOCL or AOX (order of magnitude, in kg)	Chance of by-products						
					Active chlorine	Precursors	Catalysts	High Temp.	Unexplained EOCL	Literature data	
48	AC		< 5								
49	MCA		some 3								
50	DCM		some 10								
51	Chloroform										
52	HCFCs										
53	Ethyleneamines		some 60								
54	MDI										
55	Polycarbonate										
56	Pesticides (part produced abroad)										
57	HCl		over 10	n.k.	+		often	+/-		+	
	<i>Products not produced in NL</i>										
58	PERC					+				+	
59	Trichloroethene					+				+	
60	Chlorobenzenes					+				+	
61	Chlorophenoles									+	
62	Chloroethane										
63	Chloroparaffins					+				+	

9.2 Studies facilitating the interpretation of results

Lesser-known chlorinated compounds reported from field studies

Source: OVOC report Klein et al. (2001a)

A wide variety of anthropogenic chlorinated compounds is found in the various environmental compartments. Traditional organochlorine compounds can explain only a limited part of the EOCl amount in certain environmental media. Compounds that are found vary from simple aliphatic compounds to complicated multi-heterocyclic compounds. Unfortunately, the possible sources of these compounds are not always clear. Some identified sources are the production processes for polymers, dyes, flame retardants, plasticisers and textiles, wastewater disinfection, waste incineration and the production of pulp and paper. Table 9.2.1 summarises the results of literature citations concerning lesser-known chlorinated compounds.

Role of degradation products

Source: OVOC-report Klein et al. (2001b)

Degradation pathways and products of high-emission chlorinated compounds have been identified. Degradation pathways depend on the initial emission compartment of the type of the emission (free compound or adsorbed to particles) and on such environmental conditions as temperature, intensity and the availability of solar radiation and micro-organisms. Degradation products of low-emission compounds, including a large number of emissions to surface water, were not studied. Degradation products of substances that are emitted to air and transformed in the environment can be expected to appear in other environmental compartments.

An overview of the results is given in Table 9.2.2, which contains the main degradation products of all the substances discussed in the atmosphere or soil-water system. The main purpose of this table is to give a quick overview of substances that can be detected in the environment for a long time to come. The persistence of the original substance and its degradation products is denoted qualitatively, using +, ++ or +++. A '+' sign means that the substance transforms within approximately one week, a '++' sign means that the substance transforms within several weeks, and a '+++' means that it takes months or longer for the substance to be transformed. In soil-water systems, a subdivision between aerobic and anaerobic conditions is made.

Table 9.2.1 Lesser-known chlorinated compounds reported from field studies.

Aliphatic

Group Name	Compound names of examples	Compartment	Possible source
Alkane	(1-chloro-2-propyl)(3-chloro-1-propyl)phosphate, Nitrochloroform, Tri(2-chloroethyl)phosphate, 1	Fresh water, Seawater, Human Population, Air	Flame retardant, Effluent domestic WTP, Chlorination DW & WW, Chlorobleaching of pulp
Alkene	Allylchloride, Chloroprene, Dichloroacetylene	Air	Chlorobleaching of pulp, CWI, Effluent domestic WTP
Alcohols, thiols, ethers and sulfides	Bis (2-chloroethoxy) methane, Chloromethyl methyl ether	Water/Sediment	Manufacture of polymer
Diols	Chloral hydrate		Effluent domestic WTP, Chlorination DW & WW
Alkanal	2-chloropropenal, Phosgene, Trichloroacetaldehyde, 3,3 dichloropropenal	Air	Chlorobleaching of pulp, Effluent domestic WTP, Chlorination of DW & WW
Ketons	1,1 dichloro-2- butanone, 1,1,1-trichloroacetone, Hexachloroacetone, Chloroacetone	Sediment	Chlorination DW & WW, Effluent domestic WTP, Chlorobleaching of pulp
Esters	Chlorinated methyl ester		Chlorination of DW & WW
Acids	Chloroacetates, Dichlorostearic acid	Water, Fish, Air	Chlorination of DW & WW; air emission of halocarbon
Carbohydrates and polysaccharides	Chlorolignin, Di-isopropyl chloromalonate	Sediment, Water	Chlorobleaching of pulp
Nitrile	Bromochloroacetonitrile, Dichloroacetonitrile		Effluent domestic WTP, Chlorination of DW & WW
Cyclic	3-chloro-1-propynyl-cyclohexane, Hexachloro-cyclopentadiene	Air	Effluent domestic WTP
Heterocyclic	3,5,6-trichloro-2-pyridinol, Bromochlorothiophene, Tetrachlorothiophine	Human Population, Sediment	Chlorination of DW & WW, CWI, Effluent domestic WTP
Epoxides	3,3,3-trichloroepoxypropane, Epichlorhydrin	Air	Chlorination of DW & WW, Effluent domestic

Group Name	Compound names of examples	Compartment	Possible source
Cyanides	Cyanogen chloride		WTP
Aminen	Chlorpropamide, Dichloroacetamide isomer, Monochloramine	Water, Wastewaters	Chlorination of DW & WW Chlorination of DW & WW

Aromatics

Group Name	Compound Name	Compartment	Possible source
Alcohol	Chlorophenols, Chlorocymenes, Chlorosyringols, Tetrachlorocatechol	Marine fish, Human Popula- tion, Sediment, Water	Effluent of domestic WTP, Chlorobleaching of pulp, CWI
Epoxide	Chlorostilbene oxide	Sediment/Water	
Esters	Carbochlorodithioic acid, S-methyl ester	Human Population	
Nitril	2-chloro-6-methylbenzotrile	Human Population	
Thiol	Chlorothiophenol (chlorobenzenethiol)		CWI
Keton	(Chlorophenyl)phenylmethanone, 3,4-dichloro-4'- (trifluoromethyl)benzophenone	Human Population	
Acids	2,4,6-trichlorobenzoic acid, 4-hydroxybenzoate (4- OHB)	Freshwater Sediment	Effluent of domestic WTP, CWI
Anilline	3,3 dichlorobenzidine, n-acetyl-3-chloro-p-toluidine, Dichloroaniline	Sediment, Water,	Production of pigments, Textile,
Heterocyclic	Alkylated Derivatives of Thianthrenes, Chlorothio- phenes	Water, Fish, Sediment	chlorobleaching of pulp, CWI

Group Name	Compound Name	Compartment	Possible source
Benzenes and Polybenzenes	1,2,3,4,5,6,7-heptachloronaphthalene, Chloropyrenes, Trichloroanthracene, Metabolites of PCBs	Fish, Sediment, Human Population, Water	chlorobleaching of pulp, biotransformation of PCB's, CWI
Aldehydes	2,6-dichlorosyringaldehyde, Chlorovanillins	Fish, Water	chlorobleaching of pulp
Ether	2,3,4,6-tetrachloro-anisole, 2-chloroethylvinylether, 4,5 dichloroguaiacol, Polychlorinated Diphenyl Ethers	Air, Water, Sediment	CWI, Chlorobleaching of pulp, Impurities in technical chlorophenol
Nitro, amino & cyano benzines	1-chloro-2,4-dinitrobenzene, Trichlor-1,3-dicyanobenzene, 2-amino-3-chlorophenol	Water	plasticizers/flame retardants

Pesticides & derivates

Group Name	Compound Name	Compartment	Possible source
Pesticides & Derivates	Atrazine, Toxaphene, Oxychlorane, Triclosan	Soil, Water, Air, Seal pup	

Organometallics and unclassifieds

Group Name	Compound Name	Compartment	Possible source
Organometallic	Chloromethylmercury, Lead chlorophosphate	soil	
Unclassified	2-chlorovinylarsonous acid, Chloramphenicol, Aminochlorophenoxazones	Soil, Air	

CWI = Chemical Waste Incineration;

WW = Waste Water;

DW = Drinking Water;

WTP = Wastewater Treatment Plant

Table 9.2.2 Degradation products.

Substance	In the atmosphere			In soil or water		
	Persistence of original compound	Product degradation	Persistence of product degradation	Persistence of original compound aerobic/aneerobic	Product degradation	Persistence of product degradation
Chlorophenol	?	?	?	++	chlorophenols, tetrachlororesorcinol various dimeric benzoquinones	++
1,1,2-trichloroethane	+++	phosgene, Cl ₂ , HCl, CO ₂	+	+++/>+++	Chloroacetic Acid, glyoxylic acid, Vinyl chloride	+
1,1,1-trichloroethane	+++	phosgene, Cl ₂ , HCl, CO ₂	+	+++/>+++	1,2-dichloroethane	
dichloromethane	+++	phosgene	+	+/>+	methyl chloride	+
1,1-dichloroethane	+++	formylchloride	?	?	?	?
HCFC-22	+++	HCl, CO ₂ stratospheric	?	?	?	?
Vinylchloride	++	chloroacetyl aldehyde for- mylchloride	+	+++/>+++	glycolic acid CO ₂	+
1,1-dichloroethene	+	phosgene, CO, chloroacetyl chloride	+	+++/>+++	vinylchloride	+++
1,2-dichloropropane	+	?	?	+++	?	?
1,3-dichloropropene	+	?	?	+/>?	3-chloroallyl alcohol, 3- chloroacrylic acid	?
polychlorinated biphenyl	+++	?	?	+++/>+++	3-chlorobenzoicacid, methyl-sulfonyl compounds, hy- droxy-PCB's	+++
CFC-113	+++	HCl CO ₂ (stratospheric)	?	+++/>+++	HCFC-133, HCFC-133b	?
Carbon tetra- chloride	+++	COCl ₂	+	++/>+	CCl ₄ , CS ₂	+
tetrachloroethene	+	trichloroacetic acid	++	+++	trichloroethene,	+++
trichloroethene	+	dichloroacetic acid	++	+++	1,2-dichloroethene, chloroethane, di- chloromethane	+

Explanation of persistence classification:

- + Substance transforms within approximately one week;
- ++ Substance transforms within several weeks;
- +++ Months or longer required for the substance to transform.

9.3 Publications and reports from OVOC project

Contents of CD-ROM with subproject reports.

Technical Reports Phase-1 (1999-2000)

- R1 Belfroid, A.C., Stroomberg, G.J., Letcher, R.J., Stam, G.J. & van Hattum, B. (2000). Chemical and bioscreening in search of toxic chlorine compounds in wastewater. Integrative report on the results of the pilot study OVOC. IVM Report (W-00/10), Vrije Universiteit, Amsterdam (NL).
- R2 Kleijn, R., Elshkaki, A., de Koning, A. & Tukker, A. (2001). Literature study on unknown organochlorine compounds in the environment from anthropogenic sources. CML.
- R3 Kleijn, R., Elshkaki, A., de Koning, A. & Tukker, A. (2001). Literature study on degradation products of known emissions. CML.
- R4 Tukker, A. (2000). Chlorinated micropollutants: Literature search on emissions. Backgrounds to the priority setting for the main research phase. Reportnr. STB.
- R5 Tukker, A. (2000). Chlorinated micropollutants: priority setting. Draft report of Task 3.1 and 3.2 of the preliminary investigation of the chlorine micropollutant study. Report nr. STB.

Technical Reports Phase-2 (2001-2004)

- R6 Lamoree, M.H., Stroomberg, G., Besselink, H., Senhorst, H., Sanderson, T. & van Hattum, B. (2003). *Chlorinated Micropollutants in Aquatic Effluents. Part-1 Part-1 Sampling, pre-treatment, classification and primary effect screening*. IVM Report (E-03/02), Vrije Universiteit, Amsterdam.
- R7 Senhorst, H., Lamoree, M.H., van der Linden, S., Sanderson, T. & van Hattum, B. (2004). *Chlorinated Micropollutants in Aquatic Effluents. Part-2 Biodegradation studies on selected effluents (Workpackage 8)*. IVM Report (E-04/02), Vrije Universiteit, Amsterdam.
- R8 Lamoree, M.H., Sanderson, T., Senhorst, H., Leonards, P. & van Hattum, B. (2004). *Chlorinated Micropollutants in Aquatic Effluents. Part-3 Refined fractionation and primary TIE studies*. IVM Report (E-04/01), Vrije Universiteit, Amsterdam.
- R9 Senhorst, H., Lamoree, M.H., van der Linde, S., Sanderson, T., van Stee, L.L.P. & van Hattum, B. (2004). *Chlorinated Micropollutants in Aquatic Effluents. Part-4 In-pant TIE studies (Workpackage 9)*. IVM Report (E-04/03), Vrije Universiteit, Amsterdam.
- R10 Van Hattum, B., Peters, R., Timmer, J., Swart, K., Lamoree, M.H., van der Linden, S., Leonards, P. & Felzel, E. (2004). *Chlorinated Micropollutants in Atmospheric emissions*. IVM Report (E-04/04), Vrije Universiteit, Amsterdam.
- R11 Van Hattum, B., Swart, K., Lamoree, M.H., van der Linden, S., Senhorst, H., de Voogt, P. & Tukker, A. (2004). *Chlorinated Micropollutants in Products*. IVM Report (E-04/05), Vrije Universiteit, Amsterdam.
- R12 Lamoree, M., Swart, K., Senhorst, H. & van Hattum, B. (2004). *Validation of the acidic sample clean-up procedure for the DR-CALUX assay*. IVM Report (L-04/03), Vrije Universiteit, Amsterdam.
- R13 Gerritsen, A. (2004). *Comparison of ecotoxicity of effluents from chlorine chain industry with effluents from other industrial sectors*. RIZA notanr. 2004.078X. RIZA Lelystad.
- R14 De Koning, A & Tukker, A. (2004). *Literature study on the importance of current anthropogenic PCDD/PCDF emissions in the Netherlands*. CML-IE Working Paper 2004.001. Centre of Environmental Science, Leiden University, Leiden (draft).

- R15 Tukker, A. (2004). *Measures taken on 3 Dutch industrial sites to prevent emissions of chlorinated (micro)emissions between 1985 and 2002*. Report nr. STB-04-03. TNO-STB, Delft (draft).

Meeting Reports Technical Workshops

Chlorine chain follow-up studies (OVOC) - *Meeting Report Workshop Phase-I, May 18th, 2000*. IVM Report (W-00/18), Vrije Universiteit, Amsterdam.

Van Hattum, B., Lamoree, M.H. & Stroomberg, G. (2000). *Chlorine chain follow-up studies (OVOC) - Meeting Report Workshop-1Phase-2*, Den Haag, August 30th, 2001. Report. no. W-01/xx. Vrije Universiteit, Amsterdam. 20 p.

Van Hattum, B., Lamoree, M.H., Senhorst, H., Sanderson, T. & Tukker, A. (2003). *Chlorine chain follow-up studies (OVOC). Meeting Report 3rd Technical Workshop, May 14-15, 2003*. IVM Report (E-03/13), Vrije Universiteit, Amsterdam. pp. 56.

Programme proposal and workplans (1998-2000)

Van Hattum B., Pols, H., van den Berg, M., Seinen, W., Brouwer, A., Tukker, A., Kleijn, R. & Wegener, J.W.M. (1998). *Proposal Research Programme Chlorine Chain Follow-up Studies (OVOC)*. Vrije Universiteit, Amsterdam. (English and Dutch version)

Van Hattum, B., Tukker, A., Pols, H., van den Berg, M. & Belfroid, A. (1999). *Onderzoeks programma Vervolgonderzoek Chloorketenstudie (OVOC) – Werkplan Vooronderzoek*. IVM Report (W-99/38), Vrije Universiteit, Amsterdam.

Van Hattum, B., Tukker, A., Senhorst, H., van den Berg, M., Belfroid, A. & Lamoree, M.H. (2000). *Research Programme Chlorine Chain Follow-up Studies (OVOC). Work plan Main Studies – Phase 2*. IVM Report (W-00/27), Vrije Universiteit, Amsterdam.

9.4 Summary information on DR-CALUX and Carp-Hep assays

Table 9.4.1 Main features, performance characteristics, status of validation and main applications of the DR-CALUX test and Carp-hep EROD assay, as summarised during the 3rd technical workshop, 14-5 May 2003, Den Haag.

Assay Name	DR-CALUX	Carp-Hep
Cell type	Rat hepatoma	Fresh carp hepatocytes
Response	Ah receptor-mediated luciferase induction	Ah receptor-mediated EROD (CYP1A) induction
Exposure time	24-hour exposure	96 h exposure
Sensitivity	High sensitivity	Less sensitive for some co-planar PCBs; assay reflects the sensitivity of the fish species used.
Reliability	Highly repeatable and reproducible	Repeatability good within a single carp preparation; reproducibility fair-good (dependent on the biological variability of the genetically uniform strain of carp used, and on the quality of the cell preparation process)
Metabolism	Plays limited role in 24-hour exposure	plays greater role
Validity and acceptance	Well-described and characterized. Extensively used as bio-analytical screening tool by academic laboratories and regulatory agencies, as described in the scientific literature (Garrison et al., 1996; Murk et al., 1996; Sanderson et al., 1996; Sanderson and Giesy, 1998; Behnisch et al., 2002)	Well-described and characterised in scientific literature Used on contract basis for specific aquatic environmental questions (Smeets et al., 1999a; Smeets et al., 1999b; Smeets et al., 1999c; Rankouhi et al., 2002)
Usage	Bio-analytical tool	Environmentally more relevant tool

Source: OVOC workshop report May 2003.

Table 9.4.2 Comparison between I-TEF (NATO, 1998; Van Zorge et al., 1989), WHO-TEF (Van den Berg et al., 1998) en REP values derived for the DR-CALUX® for PCDD/Fs. REP values are derived at EC50 level (derived from Benisch et al., 2002).

	I-TEF	WHO-TEF	DR-CALUX® REP (at EC ₅₀)
2378 TCDF	0.1	0.1	0.32
12378 PCDF	0.05	0.05	0.21
23478 PCDF	0.5	0.5	0.5
123478 HCDF	0.1	0.1	0.13
123678 HCDF	0.1	0.1	0.039
234678 HCDF	0.1	0.1	0.18
123789 HCDF	0.1	0.1	0.11
1234678 HCDF	0.01	0.01	0.032
1234789 HCDF	0.01	0.01	0.041
OCDF	0.001	0.0001	-
2378 TCDD	1	1	1
12378 PCDD	0.5	1	0.54
123478 HCDD	0.1	0.1	0.3
123678 HCDD	0.1	0.1	0.14
123789 HCDD	0.1	0.1	0.066
1234678 HCDD	0.01	0.01	0.05
OCDD	0.001	0.0001	-

9.5 PBT definitions in regulatory frameworks

OSPAR

The OSPAR convention for the protection of the North eastern Atlantic Ocean considers a list of substances identified for priority action (*OSPAR Summary Record 2002, OSPAR 02/21/1-E*). At present, the list mentions 46 substances or groups of substances (e.g., dioxins, PAHs). The Dynamec mechanism has been adopted for the future extension of this list. This mechanism involves a screening method that considers both hazard properties of substances and information on environmental occurrence. Criteria used in the screening method for the P, B and T properties are given in Table 9.5.1.

EU Water Framework Directive (WFD)

The EU WFD (2000/60/EC) aims to modernise the environmental protection of surface water. It transcends and integrates previous EU directives. Thereby, the WFD priority substance list encompasses e.g., the ‘black’ and ‘grey’ lists of the EU.

The COMMPS (combined monitoring-based and modeling-based priority-setting scheme) method is used to periodically extend the list of priority substances). This method combines data on monitoring in European surface waters with data on environmental characteristics and the toxicity of substances to calculate a priority index. It does not explicitly consider PBT criteria and is therefore not listed in Table 9.5.1.

SOMS

The Dutch strategy for substance management (SOMS, *Voortgangsrapportage December 2001*) developed a preliminary set of criteria for screening the most hazardous substances. Depending on the type of use in society (ranging from controlled on-site use to use in consumer products), limiting criteria are defined for P, B and T characteristics, see Table 9.5.1.

EU substance management

The **EU REACH** strategy on substance management is presently under construction. It has adopted an interim strategy on PBT substances in order to create a short-cut for managing priority substances. The PBT criteria adopted in the strategy (*ECB 4/14/02, 21 November 2002*) are given in Table 9.5.1.

UN Strategic Approach to International Chemicals Management (SAICM)

In the framework of the UN Stockholm Convention on persistent organic pollutants (POPs), 12 (groups of) substances have been named for priority action (‘the dirty dozen’): mainly pesticides and PCDDs, PCDFs and PCBs). At present, initial preparations are taking place for extending the list with the ‘SAICM mechanism’.

Table 9.5.1 PBT definitions and criteria in regulatory frameworks.

		Emission control	Substance management aiming at safe use for humans and the environment	
		OSPAR (Dynamec)	SOMS	EU-REACH interim PBT strategy
PBT if all three criteria are ful- filled	P	half-life in aquatic environ- ment ≥ 50 days	three categories between 'inherently biodegradable (adaptive or incomplete)' and 'not inherently biodegrad- able and no abiotic degrada- tion'	half-life: > 60 d (marine water) > 40 d (fresh water) > 180 d (marine sediment) > 120 d (freshwater sediment)
	B	$\text{Log } K_{ow} \geq 4$ or $\text{BCF} \geq 500$	four categories between $\text{Log } K_{ow} \geq 3$ ($\text{BCF} \geq 100$) and $\text{Log } K_{ow} \geq 5$ ($\text{BCF} \geq 500$)	$\text{BCF} > 2000$ ($\text{Log } K_{ow} \geq 4.5$) ¹
	T	aquatic toxicity: $\text{L(E)C}_{50} \leq 1$ mg/l (acute) $\text{NOEC} \leq 0.1$ mg/l (long- term) or mammalian toxicity: CMR or chronic	three categories between $\text{NOEC} \leq 1$ mg/l ($\text{LC}_{50} \leq 10$ mg/l) and $\text{NOEC} \leq 0.01$ mg/l or CMR ($\text{LC}_{50} \leq 0.1$ mg/l)	chronic $\text{NOEC} < 0.01$ mg/l or CMR or endocrine disrupting effects
vPvB if both criteria are ful- filled	vP	no criterion	see P criterion	half-life: > 60 d (marine or freshwater) > 180 d (marine or freshwater sediment)
	vB	$\text{Log } K_{ow} > 6$	see B criterion	$\text{BCF} > 5000$ ($\text{Log } K_{ow} \geq 4.5$) ¹

¹ If BCF values are not available, $\text{Log } K_{ow}$ values may be used to screen for potentially bioaccumulative substances.

9.6 Analysis of GC-MS data and identification with AMDIS-NIST

Source: OVOC reports Senhorst et al. (2004b), Van Hattum et al (2004b), Lamoree et al. (2004a)

The interpretation of GC-MS data is given schematically in Figure 9.6.1. It involves a combination of automated interpretation of GC-data with AMDIS software and a preliminary manual interpretation of AMDIS output. It seeks to find an efficient and effective screening of GC-MS data in terms of readily available chemical data of known compounds.

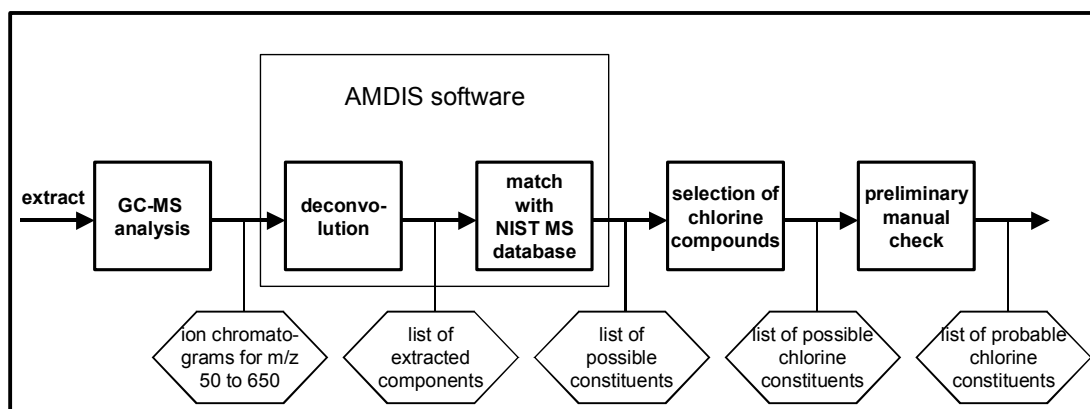


Figure 9.6.1 Schematic diagram of the interpretation of GC-MS data.

Deconvolution

Deconvolution and matching with database mass spectra is performed by AMDIS software. Deconvolution involves the extraction of structures such as peaks and ramps in any of the 600 ion chromatograms with a width of the order of 20 scans (about 10 seconds). Because of this ion mass specificity, deconvolution is able to resolve overlapping peaks or to extract peaks that are hardly visible, as they fall within a much more abundant continuum. Structures that are very much wider than 20 scans, on the contrary, have a significant probability of being overlooked by AMDIS. Deconvolution settings can vary from sensitive, which gives a larger number of extracted components at the risk of introducing false positives based on background noise, to relatively insensitive, rendering fewer extracted components that have a higher probability of being true chromatographic elements. In this study, we want to reduce the risk of false negatives (substances being overlooked) while at the same time minimising the amount of manual interpretation of AMDIS output data, which is an inevitable step in interpretation. Therefore, an optimum setting was developed here to allow a limited number of false positives.

Matching

The matching of extracted mass spectra with the NIST mass spectral database was performed automatically within AMDIS. The NIST database is the most extensive mass spectral database, containing about 100,000 mass spectra. Due to statistical error, errors introduced in the deconvolution step and apparatus-induced variation, even matches of specific known substances will never be exact. AMDIS calculates a match factor between 0 and 100, with higher values meaning better matches. Generally speaking, a

match factor of 70 is considered reasonable. Because of the large number of mass spectra in the database, AMDIS frequently arrives at several substances whose match factors exceed a certain minimum value. All AMDIS identification results should be interpreted as suggestions or likely candidates. AMDIS can at least give a hint of the direction in which substances that are actually present can be found, and may often identify the exact components directly.

AMDIS could not give a proper identification for all extracted components fulfilling the minimum match factor requirements adopted in this study. Erratic, extremely noisy mass spectra could not be matched, neither could some very well defined chromatographic peaks be interpreted. The latter phenomenon must be explained by the fact that the NIST database contains no mass spectrum that resembles that of the extracted component in any way.

Selection of chlorine containing compounds

In this study, we first screened the AMDIS output for all chlorine-containing compounds. This was done by searching for the phrase 'chlor' in the chemical name. Despite its simplicity, we consider this selection method robust. AMDIS uses a structural chemical nomenclature indicating basic functional groups, with halogens being described as such. This leaves little chance that our selection method will overlook chlorine-containing compounds.

There were two requirements for a component to be selected into our list.

First, a minimum match factor of 70 was required for the suggested chlorine-containing compound. Second, at least three of the five highest-ranking suggestions exceeding a match factor of 60 must contain chlorine.

The latter criterion is justified by the fact that, although not fully determining the exact nature of the component in such a case, ADMIS nevertheless recognizes certain structures that appear to be related to chlorine. In fact, such recognition is very likely, as chlorine-containing compounds indeed exhibit very definite structures, due to the specific isotope ratio of masses 35 and 37.

Preliminary manual check

The list of possible chlorine-containing components obtained in the manner described above is manually checked to remove artefacts introduced by AMDIS. Such artefacts could include:

- Erratically deconvoluted components: these can be due to stray data points or to random variation in the recognition of noise levels as structures;
- Incomplete deconvolution of components: due to the statistical nature of the AMDIS algorithm, which looks for structures that exceed a certain noise level; there is always a chance that certain mass lines will be included erratically or left out of the extracted mass spectrum;
- Mass lines overlooked in matching mass spectra: the matching algorithm in AMDIS simply treats all ion masses equally. It could thus overlook significant features, such as base peaks and parent ions that may give specific information on the components origin.

In this manual check, a most probable hit is determined. In case none of the suggested compounds appears to be responsible for the component that was observed, a check is made for chlorine-specific structures. This interpretation of GC-MS data is able to establish the number, and partly the nature, of components that probably contain chlorine. The interpretation should not be considered to be a full identification of chlorine-containing compounds. Neither can this interpretation be considered complete, as it can only detect compounds that are included in the NIST database. Compounds that are 'exotic' and compounds that are highly specific by-products of reactions have a fair chance of being overlooked by this interpretation scheme.

Quality aspects of identification

Generally, identification of GC-MS data is considered reliable when there is a good match on both mass spectrum and retention time. For practical reasons, the match on retention time was not performed in the OVOC study. Therefore, the identifications given should be considered as tentative identifications, in the sense that it concerns likely candidates. The procedure followed is prone to false positives as well as false negatives:

- False positives: although the probability of false positives decreases at higher match factors, even at the highest match factors (95 and higher), stereo isomers or substances of related families cannot be discerned;
- False negatives, or substances being overlooked by the identification procedure: although the NIST database contains mass spectra of over 100,000 substances, many GC-MS peaks still cannot be identified. The NIST database primarily contains substances of scientific or commercial interest. Many groups of substances, including reaction by-products and products of degradation, are poorly represented in NIST. In a number of extracts studied in OVOC, chlorinated compounds were dominantly present but still could not be identified by NIST. There is a tendency for larger or more complex molecules not to be identified by NIST. Another source of false negatives is given by peaks or structures that were too broad to be deconvoluted by AMDIS.

Manual extension of identification procedure for chlorine-containing compounds

In order to circumvent the tendency of the automated procedure to produce false negatives, a manual search was performed for chlorinated compounds. Because of the specific isotope pattern on masses 35 and 37, chlorinated compounds, especially the more highly chlorinated ones, show a distinct, toothed pattern of mass lines, separated by a distance $DM=2$. Several search strategies were followed to find chlorinated compounds:

- Chlorine-containing compounds that were automatically identified were searched for mass lines of chlorinated fragments. The ion chromatograms of these mass lines then show peaks of substances that probably contain the same chlorinated fragment. The mass spectra of these peaks were searched for new chlorine isotope structures that were used for new ion chromatograms, and so forth;
- In addition, the results of the automated identification revealed a certain regularity in the occurrence of chlorinated species. Based on this regularity, the GC-MS is searched for mass lines of other chlorinated substances. The specific chlorine isotope structure is used as a check for a peak to be truly chlorinated.

These search strategies produce an extension of the list of identified chlorinated compounds with compounds:

- That are probably chlorinated, with the probability increasing with the level of chlorination (the toothed pattern becomes more distinct at higher n);
- That provide an indication of the number (n) of chlorine atoms on a molecule. Up to n = 8, patterns of differently chlorinated compounds can clearly be discerned;
- That have a lower limit on molecular mass (m/z), by interpreting the highest observed mass as being the parent peak.

Therefore, the compounds found by the manual search are indicatively characterised as chlorinated.

9.7 In-plant TIE study

Source: OVOC report Senhorst et al. (2004b)

MCD is the largest, mixed chlorine/non-chlorine site, and it showed the strongest DR-CALUX response in the screening phase. Furthermore, it represented the largest individual DR-CALUX load (without acid clean-up, see Figure 5.5). In order to establish the contribution from chlorine activities in the load of MCD, an in-plant analysis of different internal waste water streams was performed (Work package 9) to obtain information on the origin of this load. Based on the results of Work package 9, the following conclusions, were drawn:

- Qualitatively, AOX, EOX, GC-AED loads, as well as the DR-CALUX load after acidic clean-up, originate from chlorine activities, while chronic microtoxicity, the ECD signal and the DR-CALUX response without acidic clean-up originate from non-chlorine activities. Figure 5.6 shows the mass balance of the DR-CALUX response;
- The contribution of chlorine activities to the effluent DR-CALUX load amounts to 1.5g TEQ/y. Only a small percentage of this load can be explained by PCDD/Fs. The remainder of the DR-CALUX response cannot be identified on the basis of the available information.

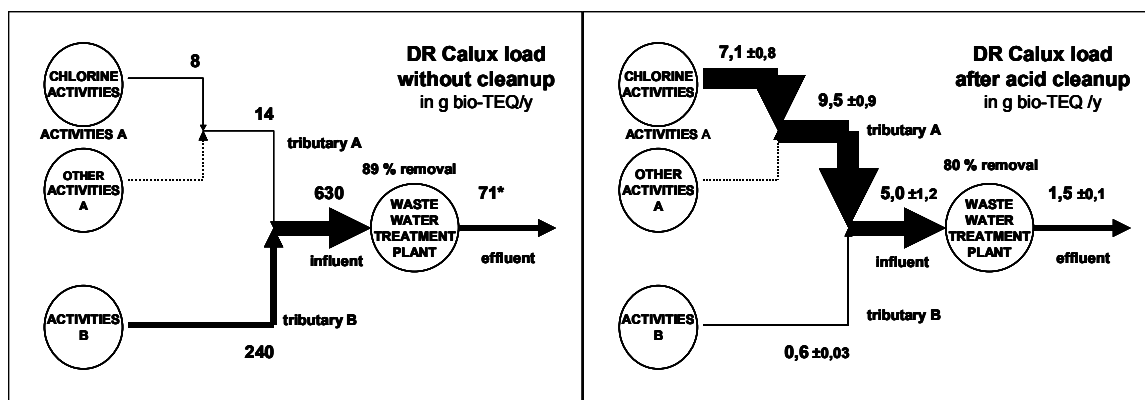


Figure 9.7.1 Comparison of DR-CALUX mass balance over influent and effluent streams at site MCD.

Note: The left panel gives the response without acidic clean-up, the right panel with acidic clean-up. The thickness of the lines is scaled to the influent DR-CALUX load, which is much lower in the right panel (5 g TEQ/y) than in the left panel (630 g TEQ/y). Results of total extracts are given.

Based on the results of the in-plant effect-directed analysis, 81% of the DR-CALUX loads (based on total extracts without clean-up) in the industrial effluents investigated can now be attributed with certainty to chlorine activities. This accuracy is considered sufficient to assess the possible impact of this load to the aquatic environment. The in-plant TIE study reduces the total industrial effluent load from chlorine activities from 21.7 to 12.2 g bio-TEQ/y (without acidic clean-up).

Comparison of the DR-CALUX response after acidic clean-up shows that the sample taken in the in-plant TIE study shows a signal that is about six times larger than that of the sample taken in the screening phase (based on coarse fractions, see Appendix 9.9).

9.8 Biodegradation study

Six effluent samples were selected to be subjected to the biodegradability test. The selection was made with toxic responses and chlorine content as positive criteria.

One of these six effluents (MAB) could not be degraded properly, due to its high salinity (100 g Cl/L).

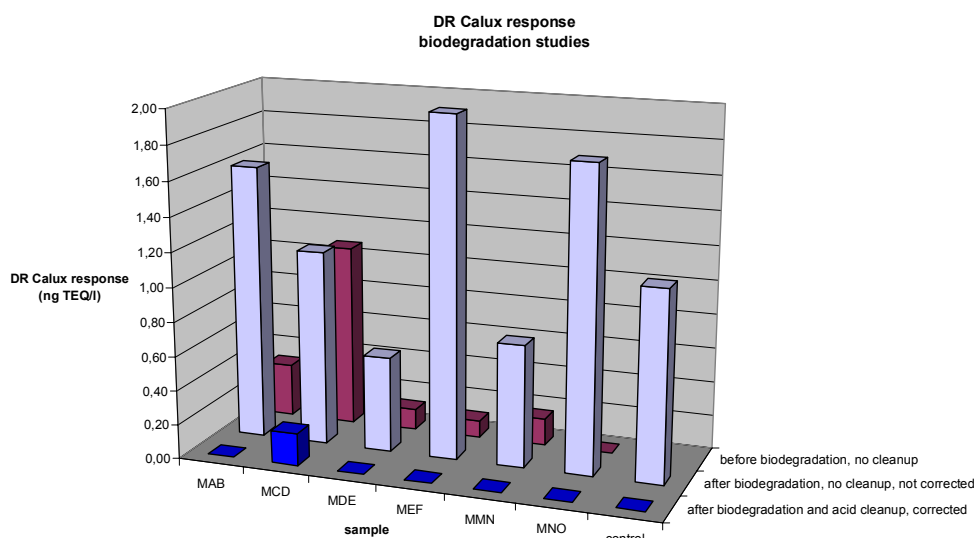


Figure 9.8.1 Results of DR-Calux response over the biodegradation test: results are given before biodegradation step (results of screening phase), after the biodegradation step without acidic clean-up and after biodegradation step with acidic clean-up applied, respectively.

Source: OVOC report Senhorst et al. (2004a)

The following results were obtained by comparison of the effluent characteristics before and after the biodegradation test:

- For one sample (MMN; municipal wastewater treatment used as a reference), reduction of chronic microtoxicity over the biodegradation test could be established. For three other samples, chronic microtoxicity still persisted after the biodegradation test. As the responses before and after the biodegradation step coincided within experimental error limits, no quantitative conclusions can be drawn with respect to possible reduction of toxicity;
- Without an acidic clean-up step, the DR-CALUX response of all samples without acidic clean-up was higher after the biodegradation test than it was in the screening phase. This phenomenon was probably explained by naturally occurring substances in the reference surface water, which were used as inoculum in the biodegradation step;
- After the application of acidic clean-up, responses to the DR-CALUX assay fell below the detection limit for most extracts. Therefore, no conclusion can be drawn regarding the biodegradability of DR-CALUX responses observed in the screening phase. Only sample MCD gave a DR-CALUX response after acidic clean-up.

Figure 9.8.1 summarises the information on the DR-Calux results over the biodegradation test.

Summarizing, the biodegradation step was not able to determine the persistence of the DR-CALUX response.

9.9 Extensive analysis of DR-CALUX response in MCD samples

Effluent MCD is the effluent that was investigated most extensively. MCD was studied in screening phase, biodegradation study, refined fractionation/primary TIE study and in the in-plant TIE study (see Figure 4.1).

For the DR-CALUX assay, this resulted in 26 measurements in several types of fractions, both with and without acidic clean-up. This section discusses the DR-CALUX responses that were obtained, to the extent that they have not been addressed in the reports of the separate studies.

It is interesting to note that:

- There is a very good match of DR-CALUX responses in the coarse fractions in screening phase (Series 1) and primary TIE study (Series 3), despite the fact that the latter series originates from a sample that was kept in cold storage for more than a year and then re-analysed. The fact that the total response of medium and high fractions differs by not more than 15% lends confidence to the comparison of DR-CALUX responses of different series;
- The response of the coarse fractions after acidic clean-up for Series 1 (screening phase) is lower by almost a factor of 6 than the corresponding values of the in-plant TIE study. Because the response after acidic clean-up in the in-plant TIE study was shown to originate entirely from the chlorine activities (see Figure 5.6), this response apparently varies with this factor between the two independent samples. This conclusion is remarkable, as other effluent parameters originating from the chlorine activities wastewater (AOX, and EOX) do not significantly differ between the two samples;
- The response of the fine fractions after acidic clean-up (Series 3) was a factor of 20 below the response of the coarse fractions after acidic clean-up (Series 1). Apparently, even after the application of acidic clean-up, refined fractionation (and thereby probably metabolism), was able to lower the response by an order of magnitude. This result coincides with the conclusion of acidic clean-up step in the validation study, which shows that non-persistent substances may survive the clean-up step.

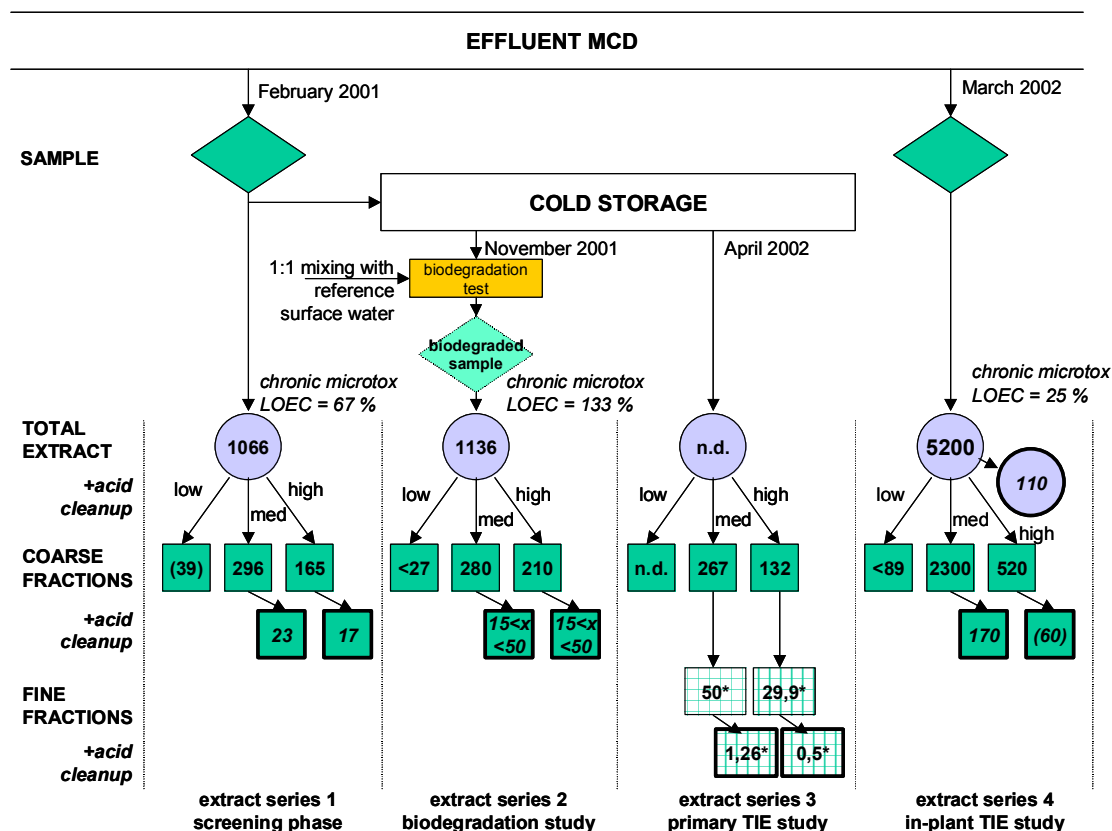


Figure 9.9.1 An overview of all DR-CALUX responses obtained with extracts of effluent MCD.

Note: Values given are in pg/l. Application of acidic clean-up is denoted by bold perimeters of figures; values given in parentheses were below the Limit of Quantification (LOQ) and above the Limit of Detection (LOD); * denotes the sum of responses of fine fractions investigated; results after biodegradation have not been corrected for 1:1 dilution;

Source: based on OVOC reports Lamoree et al. (2003, 2004a), Senhorst et al (2004a, 2004b).

9.10 Glossary (abbreviations)**Institutions / commissions**

Abbr.	In Dutch	In English
Ministry VROM	Ministerie van Volkshuisvesting, Ruimtelijke Ordening en Milieubeheer	Ministry of Housing, Spatial Planning, and the Environment
Ministry V&W	Ministerie van Verkeer en Waterstaat	Ministry of Transport, Public Works, and Water Management
RWS	Rijkswaterstaat	Directorate General of Public Works, and Water Management
RIZA	Rijks Instituut voor Integraal Waterbe- heer en Zuivering van Afvalwater	National Institute for Inland Water Management and Waste Water
VNCI	Vereniging Nederlandse Chemische Industrie	Association of the Dutch Chemical Industry
IRAS		Institute for Risk Assessment Studies, Utrecht University
TNO-STB	Nederlandse Organisatie voor Toege- past Natuurwetenschappelijk Onder- zoek – Strategie, Technologie en Be- leid	TNO Strategy, Technology and Policy Studies
IVM	Instituut Voor Milieuvraagstukken, Vrije Universiteit	Institute for Environmental Studies, Vrije Universiteit
CML	Centrum voor Milieukunde Leiden, Universiteit Leiden	Centre for Environmental Sciences, Leiden University
BITAC	Begeleidingscommissie Implementatie Acties Chloorketenstudie	Committee for the Implementation of Actions of the Chlorine Chain Study
OVOC	Onderzoeksprogramma Vervolgonder- zoek Chloorketenstudie	Research Programme Chlorine Chain Follow-up Studies
BOVOC	Begeleidingscommissie Onderzoeks- programma Vervolgonderzoek Chloor- ketenstudie	Supervising Committee Research Programme Chlorine Chain Follow-up Studies
SNM	Stichting Natuur en Milieu	The Netherlands Society for Nature and Environment
SENSE	Onderzoeksschool Sociaal Economisch en Natuurwetenschappelijk Milieuon- derzoek	Research Graduate School Socio- Economic and Natural Sciences of the Environment

Technical terms

Abbr.	In Dutch	In English
AED	atomaire emissie detectie	atomic emission detection
ECD	electronen invang detectie	electron capture detection
ELS		early life stage test
GC	gas chromatografie	gas chromatography
HCH	hexachloorcyclohexaan	hexachlorocyclohexane
HCl	zoutzuur	hydrochloric acid
HPLC	hoge druk vloeistof chromatografie	high pressure liquid chromatography
HPLC	hoge druk vloeistofchromatografie	high pressure liquid chromatography
HRGCMS	hoge resolute gaschromatografie in combinatie met hoge resolutie massa-spectrometrie (voor analyse van dioxines)	high resolution gas chromatography with high resolution mass spectrometry (used for analysis of PCDD/Fs)
<i>in vitro</i>	test of experiment met orgaan, weefsel of celkweek van organisme	test or experiment with organ, tissue or cell culture of organism
<i>in vivo</i>	test of experiment met levend en intact organisme	test or experiment with living whole organism
ITD		ion trap detection
ITD	ionen trap detectie	ion trap detection
I-TEQ	totale toxische equivalente concentratie berekend op basis van gemeten concentraties PCDD/Fs en internationale TEF waarden voorgesteld door NATO/CCMS in 1988	total toxic equivalent concentrations derived from measured concentrations of PCDD/Fs and international TEFs proposed by NATO/CCMS in 1988
K_{ow}	n-octanol/water partiticoëfficiënt	n-octanol/water partitioning coefficient
K_{ow}	n-octanol/water partiticoëfficiënt	n-octanol/water partitioning coefficient
LC50	mediaan lethle concentratie	median lethal concentration
LCA	levensloop analyse	life-cycle analysis
LDH	lactaatdehydrogenase	Lactate dehydrogenase
MCA	monochloorazijnzuur	monochloro acetic acid
Microtox	micri-toxiciteitstest met luminescerende mariene bacterie <i>Vibrio Fischeri</i>	micro-toxicity test with luminescent marine bacteria <i>Vibrio Fischeri</i>
MS	massa spectrometrie	mass spectrometry
MSD	massa spectrometrische detectie	mass spectrometric detection
MSD	massa spectrometrische detectie	mass spectrometric detection
MSWI	gemeentelijke afvalverbrandingsinstallatie	municipal solid waste incinerator
MTT	test voor mitochondriale dehydrogenase activiteit	test for mitochondrial dehydrogenase activity, using 3-(4,5-dimethyl-thiazol-2yl)-2,5-diphenyltetrazolium bromide, as substrate

Abbr.	In Dutch	In English
MWTP	gemeentelijke rioolwaterzuiveringsinstallatie	municipal wastewater treatment plant
NIST		National Institute of Standards and Technology, Gaithersburg, MD, USA
N-PAC	stikstofhoudende PAC	nitrogen
OSPAR	Oslo and Parijs Conventie	Oslo and Paris Conventions, for the protection of the Marine Environment of the North-East Atlantic
PAC	polycyclische aromatische verbinding	polycyclic aromatic compound
PAH	polycyclische aromatische koolwaterstof	polycyclic aromatic hydrocarbon
PBB	polybroom bifenyl	polybrominated biphenyl
PBDE	polybroom difenylether	polybrominated diphenylether
PBT	persistente, bioaccumulatieve en toxische verbinding	persistent, bioaccumulative, and toxic compound (see Appendix 9.5)
PCB	polychloor biphenyl	polychlorinated biphenyl
PCDD	polychloor dibenzodioxine	polychlorinated dibenzodioxin
PCDF	polychloor dibenzofurane	polychlorinated dibenzofuran
PCN	polychloor naftalene	polychlorinated naphthalene
PER	perchloorethyleen, tetrachloorethyleen	perchloroethylene, tetrachloroethylene
POP	persistente organische verontreiniging	persistent organic pollutant
QSAR	kwantitatieve structuur- en activiteits relatie	quantitative structure activity relationship
REACH	Registratie, evaluatie en Autorisatie van Chemicaliën, voorstel van EC voor nieuw beoordelingssysteem van stoffen	Registration, Evaluation and Authorization of Chemicals, new regulatory framework for evaluation of chemicals, proposed by EC
REP	relatief effect inducerend vermogen	relative effect potency
RIKZ	Rijksinstituut voor Kust en Zee	
RP-HPLC	omgekeerde fase hoge druk vloeistofchromatografie	reversed phase high pressure liquid chromatography
SAICM		
SFA	stofstroom analyse	substance-flow analysis
SOMS	Strategienota Omgaan met Stoffen	Strategie on Management of substances
S-PAC	zwavelhoudende PAC	sulphur containing PAC
TEF	toxische equivalentie factor	toxic equivalent factor
TEQ	toxische equivalente concentratie, ten opzichte van 2,3,7,8-TCDD	toxic equivalent concentration, relative to 2,3,7,8-TCDD
TEQ, bio-TEQ	totale toxische equivalente concentratie gebaseerd op meting met een bioassay	total toxic equivalent concentration based on measurement with bioassay
TEQ, I-TEQ	totale toxische equivalente concentratie berekend op basis van gemeten concentraties PCDD/Fs en internationale TEF waarden voorgesteld door NATO/CCMS in 1988	total toxic equivalent concentrations derived from measured concentrations of PCDD/Fs and international TEFs proposed by NATO/CCMS in 1988

Abbr.	In Dutch	In English
TEQ, WHO-TEQ	totale toxische equivalente concentratie berekend op basis van gemeten concentraties PCDD/Fs en nieuwe TEF waarden voorgesteld door een WHO werkgroep in 1998	total toxic equivalent concentrations derived from measured concentrations of PCDD/Fs and updated and reviewed TEFs proposed by a WHO working group in 1998
TIE	toxiciteit en identiteit evaluatie	toxicity identity evaluation
TRI	trichloorethyleen	trichloroethylene
UN	Verenigde Naties	United Nations
vPvB	zeer persistent en zeer bioaccumulatief	very persistent and very bioaccumulative
VTG	vitellogenine	Vitellogenin
WFD	Kaderrichtlijn Water (EC)	Water Framework Directive (EC)
WHO	Wereld Gezondheids Organisatie	World Health Organisation
WHO-TEQ	totale toxische equivalente concentratie berekend op basis van gemeten concentraties PCDD/Fs en nieuwe TEF waarden voorgesteld door een WHO werkgroep in 1998	total toxic equivalent concentrations derived from measured concentrations of PCDD/Fs and updated and reviewed TEFs proposed by a WHO working group in 1998

9.11 BOVOC steering committee participants

Ministry of Housing, Spatial Planning, and the Environment

- Dr. J. van der Kolk (chairman)
- Mrs. Dr. M.E.J. van der Weiden (co-ordination, until October 2002)
- Ing. M.J.G.A. Adams (co-ordination, after September 2002)

Ministry of Transport, Public Works, and Water Management

- Ir. J.P. van Dalen (until December 2002)
- Dr. P.G.J. de Maagd (after November 2002)

Association of the Dutch Chemical Industry (VNCI)

- Dr. J. Bouwma

Akzo Nobel Base Chemicals bv

- Mrs. Ir. L.A.M. van de Mast-Nijhuis

Netherlands Society for Nature and Environment (SNM)

- Drs. M. Koene

TNO Strategy, Technology and Policy

- Dr. A. Tukker (research team)

Institute for Environmental Studies, Vrije Universiteit

- Dr. B. van Hattum (project manager research team)

9.12 List of Tables and Figures

List of Tables

Table 1.1	Organisation and timetable of the OVOC project	6
Table 2.1	Aquatic and atmospheric emission sources, prioritised for OVOC study	10
Table 2.2	Products prioritised in OVOC study	12
Table 2.3	Estimated annual emissions of dioxins (PCDD/Fs in g I-TEQ/yr) in the Netherlands from recent national and European emission inventory studies	13
Table 5.1	Results of the analysis of the product samples by tentative identification as well as indicative characterisation	38
Table 5.2	Measured PCDD/F concentrations in selected samples: comparison with total DR-CALUX response (after acidic clean-up) and response attributable to the contribution of PCDD/Fs	46
Table 5.3	Atmospheric emissions of chlorinated compounds detected in fractionated extracts of emission samples with GC-NCI-MS	49
Table 5.4	Atmospheric emissions. Estimation of annual loads of dioxins determined with HRGCMS and total dioxin-like activity determined with DR-CALUX (including sulphuric acid clean-up)	50
Table 5.5	Concentrations and estimated annual loads of chlorinated impurities identified or characterised in products	50
Table 5.6	Estimation of annual loads of bioassay-based bio-TEQs via selected products	51
Table 5.7	Environmentally relevant characteristics of substances that were tentatively identified in atmospheric emissions and products of the chlorine chain in the Netherlands	53
Table 5.8	Comparison of OVOC-identified worst-case emissions with known emissions in the Netherlands to municipal sewer systems or air	57
Table 6.1	Comparison of DR-CALUX-based annual loads (after acidic clean-up and/or fractionation; in g bio-TEQ/yr) for selected sources in the OVOC project with data from recent dioxin-emission inventories (in g I-TEQ/yr)	68
Table 9.1.1	Production processes in the Dutch chlorine chain	91
Table 9.1.2	Products in the Dutch chlorine chain (produced and/or imported)	92
Table 9.1.3	Other processes (use of active chlorine as disinfectant and waste incineration)	92
Table 9.1.4	Basic data for priority setting in the main research study on chlorinated micropollutants	95
Table 9.2.1	Lesser-known chlorinated compounds reported from field studies	100
Table 9.2.2	Degradation products	103
Table 9.4.1	Main features, performance characteristics, status of validation and main applications of the DR-CALUX test and Capr-hep EROD assay, as summarised during the 3 rd technical workshop, 14-5 May 2003, Den Haag	106
Table 9.4.2	Comparison between I-TEF (NATO, 1998; Van Zorge et al., 1989), WHO-TEF (Van den Berg et al., 1998) en REP values derived for the DR-CALUX® for PCDD/Fs. REP values are derived at EC50 level (derived from Benisch et al., 2002)	107
Table 9.5.1	PBT definitions and criteria in regulatory frameworks	109

List of Figures

Figure 1.1	Summary of aggregated fluxes of chlorine in the Netherlands	3
Figure 1.2	Potential sources of organochlorine compounds in the environment	4
Figure 2.1	Total PCDD/PCDF emissions to air in the Netherlands (1970-2000)	14
Figure 3.1	Conceptual scheme of target compounds in the present study	17
Figure 3.2	General approach of OVOC study: identification and toxicity screening	18
Figure 4.1	Framework of effect-directed biological and chemical screening methods applied in the OVOC project	27
Figure 4.2	Comparison of results of tentative identification (triangles) and indicative characterization (circles) of chlorinated compounds for the analysis of trichloroethylene	34
Figure 4.3	Relation between the highest molecular mass (m/z) observed in the mass spectra of chlorinated components in the sample of trichloroethylene as a function of retention time	34
Figure 5.1	Classification of in vivo and in vitro toxicity of extracts of aquatic effluents	40
Figure 5.2	Results of detailed RP-HPLC fractionation studies and application of acidic clean-up for the 4 responsive effluents	41
Figure 5.3	DR-CALUX response of atmospheric emissions (pg bio-TEQ/Nm ³). MWSI denotes the municipal solid waste incinerator	44
Figure 5.4	DR-CALUX response measured in product extracts (pg-bio TEQ/g).	45
Figure 5.5	DR-CALUX loads (g bio-TEQ/yr), as determined for the chlorine-chain effluents in the OVOC study	48
Figure 5.6	Conceptual scheme of qualitative ranking of the DR-Calux response in samples after refined fractionation (determinations in fractions with medium and high hydrophobicity) and after acidic clean-up	60
Figure 6.1	General layout of an industrial production process in the chlorine industry, indicating the various ways in which by-products that are formed are removed from process streams such that minimal emissions to the environment occur	66
Figure 9.1.1	The Dutch chlorine chain in 1990 (values in ktpa)	89
Figure 9.1.2	Legend for substance-flow diagram (Figure 9.1.1)	90
Figure 9.6.1	Schematic diagram of the interpretation of GC-MS data	110
Figure 9.7.1	Comparison of DR-CALUX mass balance over influent and effluent streams at site MCD	114
Figure 9.8.1	Results of DR-Calux response over the biodegradation test: results are given before biodegradation step (results of screening phase), after the biodegradation step without acidic clean-up and after biodegradation step with acidic clean-up applied, respectively	116
Figure 9.9.1	An overview of all DR-CALUX responses obtained with extracts of effluent MCD	119

This report presents the overall results of the Dutch research programme on emissions of chlorinated organic microcontaminants, i.e. unexpected chlorinated compounds with persistent, bioaccumulative and toxic (PBT) properties, originating from the Dutch chlorine chain. The main objectives of the programme were to assess whether and if so, to what extent the Dutch chlorine chain emits chlorinated organic microcontaminants with PBT properties and to contribute to the environmental impact assessment of these compounds.

Research team

IVM (Institute for Environmental Studies, Vrije Universiteit, Amsterdam; co-ordination)

RIZA (Institute for Inland Water Management and Waste Water Treatment, Ministry of Transport, Public Works and Water Management, Lelystad)

TNO-STB (Netherlands Organisation for Applied Scientific Research – Strategy, Technology and Policy, Delft)

IRAS (Institute for Risk Assessment Sciences, University of Utrecht)

CML (Institute of Environmental Science, University of Leiden).

Commissioned by

Ministry of Housing, Spatial Planning and the Environment (VROM, DGM)

Ministry of Transport, Public Works and Water Management (V&W, Directorate General for Water Affairs)

Association of the Dutch Chemical Industry (VNCI)

Netherlands Society for Nature and Environment (SNM).

Cover design

mixedmedia, Bussum