# National Dioxins Program

**Technical Report No. 11**
Ecological Risk Assessment of Dioxins in Australia

**Prepared by Robyn Gatehouse**

**Australian Government**
**Department of the Environment and Heritage**

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Department of the Environment and Heritage

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**Phone:** 1800 803 772
**Fax:** (02) 6274 1970
**E-mail:** dioxins@deh.gov.au
**Mail**
National Dioxins Program
c/- Chemical Policy
Department of the Environment and Heritage
GPO Box 787
CANBERRA ACT 2601
AUSTRALIA
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# Foreword

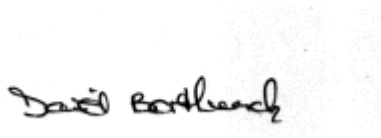
When the Australian Government established the four year National Dioxins Program in 2001, our knowledge about the incidence of dioxins in Australia was very limited.

The aim of the program was to improve this knowledge base so that governments were in a better position to consider appropriate management actions. Starting in mid 2001, a range of studies were undertaken which involved measuring emissions from sources such as bushfires, as well as dioxin levels in the environment, food and population. The findings of these studies were used to shed light on the risk dioxins pose to our health and the environment.

This work has been completed and the findings are now presented in a series of twelve technical reports.

Having good information is essential if there is to be timely and effective action by governments; these studies are a start. Our next step is to foster informed debate on how we should tackle dioxins in Australia, as this is an obligation under the Stockholm Convention on Persistent Organic Pollutants. The Department of the Environment and Heritage will be working closely with other Australian Government, State and Territory agencies to take this step.

Ultimately, the effective management of dioxins will be the shared responsibility of all government jurisdictions with the support of the community and industry.



David Borthwick
Secretary
Department of the Environment and Heritage

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- the Department of Agriculture, Fisheries and Forestry, who assessed the levels of dioxins in agricultural commodities

- Food Standards Australia New Zealand and the Department of Health and Ageing and who assessed the levels of dioxins in foods and assessed the health effects of dioxins

- officers of the Chemical Assessment Section in DEH who assessed the ecological effects of dioxins

- members of the National Dioxins Project Team which included representatives from the State and Territory environment protection agencies, the Australian Health Ministers Conference and the Primary Industries Ministers Council

- members of the National Dioxins Consultative Group which included representatives from industry and agricultural sectors, environment and public health groups and research institutions.

The Department would also like to especially thank Dr Heidelore Fiedler (UNEP Chemicals, Switzerland) and Dr Patrick Dyke (PD Consulting, United Kingdom) who provided valuable review on an early draft of this report.

# Project Team

Robyn Gatehouse and Jack Holland – Chemical Assessment Section, Department of the Environment and Heritage

# Executive Summary

This ecological risk assessment is a component of the National Dioxins Program initiated by the Australian Government to assess the impact of polychlorinated dibenzo-p-dioxins (PCDD), polychlorinated dibenzofurans (PCDF) and "dioxin-like" coplanar polychlorinated biphenyls (PCB) on Australian native fauna exposed in aquatic and terrestrial environments to ambient levels.

PCDDs, PCDFs and coplanar PCB, collectively called "dioxins" in this report, are among a group of twelve persistent organic pollutants, which were identified in the Stockholm Convention Treaty on Persistent Organic Pollutants (POPs), (2001) as priority substances for elimination or restriction of release worldwide. The 2001 Stockholm Convention on Persistent Organic Pollutants (POPs) entered into force on May 17, 2004, marking the start of international efforts to rid the world of dioxins, furans, PCBs and nine pesticides.

Dioxins are aryl hydrocarbon receptor agonists, which cause a wide spectrum of adverse toxic effects in many vertebrate species. They are particularly potent developmental toxicants at low concentrations and can disrupt the development of the endocrine, reproductive, immune and nervous system of the offspring of fish, birds and mammals when exposed from conception through postnatal or post hatching stages. In addition to their high toxicity, dioxins are widespread environmental contaminants, found worldwide, even at remote locations. They are resistant to biological and chemical breakdown, and have the ability to bioaccumulate in organisms.

The risk assessment comprises three main parts: the hazard assessment, the exposure assessment, and the risk charatcerisation. The hazard assessment was conducted using existing published studies examining the toxic effects of dioxins, which are available for a limited number of test species and classes of organisms. Toxicity reference values (TRVs) derived from these studies were adopted to assess the potential risk to native wildlife, for which no toxicity data is available. The exposure assessment was based on the results of the National Dioxin Program's data gathering surveys where dioxin levels were measured in soil, sediment and fauna at locations representative of airsheds and catchments throughout Australia. The risk characterisation was performed by combining information from the hazard and exposure assessments. The findings are summarised as follows:

- Low levels of dioxins were found in Australian soil, sediment and fauna samples, although the levels were highly variable, particularly in fauna. Dioxin levels in soil were on average highest in urban and industrial areas, while dioxin levels in sediment were on average highest in urban and industrial estuaries
- The levels in fauna reflect the animal's position in the food chain, with high trophic level aquatic and terrestrial organisms having the highest levels of dioxins in their bodies relative to other organisms in the same environment. Low trophic level herbivores accumulated the lowest levels relative to other organisms in the same environment. On average, birds of prey had the highest levels found in any organism

- PCDD/PCDFs and PCBs contributed equally to the toxic load in birds and terrestrial mammals, while for marine mammals, PCBs contributed over 90% of the toxic load in dolphins and the seal, and over 80% in whales
- The most sensitive toxicity endpoints (or toxicity reference values, TRVs) with ecological relevance applicable to this risk assessment were taken to be the no-observed-adverse-effect-level (NOAEL) for reproductive and developmental effects in early life-stage fish, birds, and mammals
- A low risk to fish was indicated from exposure to ambient dioxin levels found in the Australian aquatic environment, when using the NOAEL for embryo mortality in the most sensitive fish species tested in the laboratory
- A low risk to terrestrial mammals was indicated from exposure to ambient levels of dioxins when assuming TRVs derived for placental mammals (i.e. rats) exposed during gestation. However, the absence of data on the toxicity of dioxins to native marsupials and monotremes add significant uncertainties to this risk determination. The ramifications of the differing reproduction strategies between placental mammals and marsupials for dioxin exposure at sensitive life stages are not known
- Based on a very small data set and limited toxicity information, a potential risk is indicated for the two dolphins living in the vicinity of urban/industrial estuaries, which had higher TEQs in their bodies than mammals living in the open ocean. Tissue TEQ levels in these animals were within the threshold range TRVs found to cause toxic effects in laboratory and semi-field studies with mink, seal, and otters, which are the only aquatic mammalian wildlife for which TRVs are available. The risks to dolphins in other regions and dwelling in the open ocean are not known.
- No risk is indicated for marine mammals living in the open ocean environments of Australia, which had low levels of dioxins in their bodies
- Recognizing the inherent uncertainties in the models and assumptions, the data is sufficient to signal a potential risk to raptors exposed to ambient levels of dioxins, at least in the regions and subpopulations of birds from where the samples with the highest dioxins loads were collected. Approximately 30% of egg exposure concentrations were higher than the TRV for the most sensitive species - the domestic chicken, while approximately 20% of egg exposure concentrations were above the threshold for the bald eagle, and about 10% of eggs were above the threshold for embryo mortality in the American kestrel. Birds with the highest dioxin loads were collected in urban environments.

All risk assessments have uncertainties associated with them. The uncertainties arise from the inevitable knowledge and data gaps, which require the adoption of assumptions to cover these gaps. The above risk estimations are no exception. The conclusions are based on a small fauna data set, comprising a limited number of species and trophic levels, and whose sensitivity to the toxic effects of dioxins is not known. These inherent uncertainties should

be taken into account when interpreting the results of the risk assessments. A conservative approach has generally been adopted at all stages of the risk assessment to prevent underestimation of the risk, and this should also be kept in mind when interpreting the results.

More reliable risk estimations would require information on the toxicity of dioxins to Australian wildlife species. Australian ethical committees and current State Government legislation generally do not allow toxicity testing on native species. More targeted sampling of the eggs of raptors and other high trophic level birds, in association with field population studies of potentially exposed bird populations, would help to clarify whether dioxins are having a real impact on wild bird populations.

# Glossary/Abbreviations

| Term | Definition |
|---|---|
| Adverse response | The change in morphology, physiology, growth, development or life span of an organism which results in impairment of functional capacity or impairment of capacity to compensate for additional stress or increase in susceptibility to the harmful effects of other environmental influences. Some adaptive changes are not generally considered to be adverse e.g. some changes in enzyme levels. |
| Ah receptor | Aryl hydrocarbon (Ah) receptor is a cell protein that initiates many of the effects of dioxin-like chemicals. Its primary function in the body is uncertain, and it is structurally related to many other important cell proteins involved for instance in rhythmic functions (clock proteins) and organ development. |
| Bioconcentration | The process by which there is a net accumulation of a xenobiotic chemical directly from the abiotic environment (e.g. water) into organisms, resulting from simultaneous uptake (e.g. by gill or epithelial tissue) and elimination (e.g. via gills, faces, skin) and metabolic transformation. |
| Bioconcentration factor (BCF) | The concentration ratio ($C_o/C_w$) of the chemical in an organism ($C_o$) and in ambient water ($C_w$). |
| Bioaccumulation | The process of accumulation of chemicals in the tissue of organisms through any route, including respiration, ingestion, or direct contact with contaminated water, sediment, soil, air and pore water in the sediment or soil. |
| Bioaccumulation factor (BAF) | Defined in the same way as the BCF, but contains additional terms for dietary and other exposure routes. |
| Biomagnification | Biomagnification is usually defined as the transfer of a xenobiotic chemical from food to an organism that results in a higher concentration within the organism than the source food. The term implies an efficient transfer of chemical from food to consumer, so that residue concentrations increase systematically from one trophic level to the next. |
| Biomagnification factor (BMF) | The ratio $BAF_n/BAF_{n-1}$ or the chemical concentration in a predator organism relative to its prey (BMFs are also called predator prey factor PPF). |
| Biomarker | Any measurement reflecting an interaction between a biological system and an environmental agent, which may be chemical, physical or biological. Often used to describe measurements used in biological monitoring. |

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| Biota-sediment accumulation factor (BSAF) | The biota-sediment accumulation factor is the concentration in an organism compared to the concentration in soil or sediment, usually normalised to the lipid content of the organism and the organic carbon content in the soil or sediment. |
| Congener | One of a number of closely related chemicals derived from the same parent compound. |
| CYP1A | Cellular protein involved in the metabolism of polycyclic aromatic hydrocarbons. |
| Dioxins | The group of persistent chlorinated chemical compounds, polychlorinated dibenzodioxins (PCDDs), which share certain similar chemical structures, properties and biological characteristics, including toxicity. For the purpose of the National Dioxins Program the term "dioxins" is used in the broader sense and is also taken to include the closely related polychlorinated dibenzofurans (PCDFs or furans) and co-planar polychlorinated biphenyls (PCBs). There are several hundred of these compounds, or congeners, of which 29 are considered by the WHO to have significant toxicity. |
| Dose | A term referring generically to the amount of chemical to which an organism is exposed by any of several routes. Specifying the routes within the environmental context and especially the point of measurement is made possible via subcategories of dose (e.g. potential dose, applied dose, absorbed dose, internal dose, and delivered dose). Dose is normally expressed as a mass per unit body weight per unit time and is frequently expressed in units of mg/kg/day. |
| Dose additivity | Where a combination of two or more biologically active or toxic chemicals has an effect that is the sum of their individual effects. |
| Dose-response assessment | Estimation of the relationship between dose, or level of exposure to the substance, and the incidence and severity of an effect, where appropriate. |
| Ecotoxicology | A multidisciplinary field of study that was developed to deal with the interactions, transformation, fate and effects of natural and synthetic chemicals in the biosphere. The field incorporates concepts from disciplines such as toxicology, biology, physiology, ecology, genetics, microbiology, biochemistry, immunobiology, molecular biology, analytical, organic and environmental chemistry, soil, water and air sciences, engineering and economics. |
| Endpoint | An observable or measurable biological event used as an indicator of the effect of a chemical on a biological system (cell, organ, organism etc.). |

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| EROD | Ethoxyresorufin-*O*-deethylase, an enzyme involved in the metabolism of polycyclic aromatic hydrocarbons. |
| Exposure | A measure of the environment leading to a dose. Exposure is quantified as the concentration of the agent in the medium in contact, integrated over the duration of the contact. |
| Exposure assessment | Estimation of the concentration/dose to which human populations or environmental compartments (e.g. aquatic environment, terrestrial environment, atmosphere) are or may be exposed. |
| Exposure route | The way a chemical enters an organism after contact e.g. by ingestion, inhalation, or dermal absorption. |
| Hazard identification | Indication of the adverse effect(s), which a chemical has an inherent capacity to cause. |
| $K_{ow}$ | Octanol-water partition coefficient is defined as the concentration of a chemical at equilibrium in octanol divided by the concentration of the chemical at equilibrium in water. Octanol is a solvent that resembles lipids or organic carbon in soil. The degree to which a chemical dissolves in octanol is a measure of its lipid solubility and bioaccumulation potential. |
| $K_{oc}$ | Soil adsorption partition coefficient. A measure of the degree to which a soil will adsorb or partition to organic matter in soil or sediment. |
| $LD_{50}$ (or $LC_{50}$) | Median lethal dose or concentration. The dose or concentration of a chemical that is estimated to be fatal to 50% of those organisms exposed under the stated test conditions. |
| Lipids | One of the principal classes of macromolecules in our body, the others are proteins and carbohydrates. Lipids include fats and oils (triglycerides), fatty acids, waxes, steroids, phospholipids, glycolipids and lipoproteins. |
| Lowest observed adverse effects level (LOAEL) | The lowest dose or concentration of a toxicant that causes a significant increase in the frequency or severity of an adverse effect when compared to the frequency or severity of the same effect in an unexposed control population. |
| Microgram | $\mu g = 10^{-6}$ gram (0.000 001 g) |
| Nanogram | $ng = 10^{-9}$ gram (0.000 000 001 g) |
| No observed adverse effects level (NOAEL) | The highest dose or concentration level of a toxicant at which the incidence of a toxic effect was not significantly different from the untreated group (from a statistical and biological assessment). The NOAEL will depend on the sensitivity of the methods used, the sizes of the exposed groups and the differences between estimated exposures or doses. The NOAEL is an observed value that does not take into account the nature or steepness of the dose. |

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| PCB | Polychlorinated biphenyls |
| PCDD | Polychlorinated dibenzodioxins |
| PCDF | Polychlorinated dibenzofurans |
| Picogram | pg = $10^{-12}$ gram (0.000 000 000 001 g) |
| QSAR | Quantitative Structure-activity relationships are mathematical models used to predict specific chemical behaviour (e.g. toxicity, fate) in the environment. They may be used when measured data are not available. The models are based on the use of non-empirical structural descriptors and empirical parameters (e.g. octanol/water partition coefficient) of the chemical. |
| Receptor species | Any species or organism exposed to the chemical stressors and potentially at risk from the exposure. |
| Risk assessment | The process of estimating the potential impact of a chemical, physical, microbiological or psychosocial hazard on a specified human population or ecological system under a specific set of conditions and for a certain timeframe. |
| Risk characterization | The estimation of the incidence and severity of the effects likely to occur in a human population or environmental compartment due to actual or predicted exposure to a chemical. |
| TCDD | 2,3,7,8-Tetrachlorodibenzo-p-dioxin, the most toxic dioxin. |
| Toxic Equivalency Factors (TEFs) | See Toxic Equivalent TEQ. |
| Toxic Equivalent (TEQ) | Since dioxins occur as complex mixtures of congeners in most environmental media (air, water, soil), the concept of toxic equivalents (TEQs) has been developed. This concept allows the toxicity of a complex mixture to be estimated and expressed as a single number. Available animal-based toxicological data have been used to generate a set of weighting factors, each of which expresses the toxicity of a specific congener in terms of its equivalent mass of TCDD (2,3,7,8-Tetrachlorodibenzo-p-dioxin). Multiplication of the mass of the congener by its weighting factor (or toxic equivalency factor, TEF) yields the corresponding TCDD mass (or TEQ). The total toxicity of any mixture is then simply the sum of the individual congener TEQs. |
| Toxic Equivalent Concentration (TEC) | Same as TEQ. The TEC in environmental samples (water, soil, organisms) is calculated by analytically determining the levels of each congener in an environmental compartment, multiplying each congener by their respective TEF value, and adding all products to give a 2,3,7,8-TCDD equivalent toxicity. |
| Toxicity reference value (TRV) | The toxicity endpoint (usually derived from toxicity tests with laboratory animals) which is used to compare to exposure levels to |

determine if there is an ecological risk or not. In screening level risk assessment, the TRV is typically the lowest ecologically relevant toxic endpoint for the most sensitive test species.

# Contents

**ACKNOWLEDGEMENTS** ..... **IV**

**EXECUTIVE SUMMARY** ..... **V**

**GLOSSARY/ABBREVIATIONS** ..... **VIII**

**1 INTRODUCTION** ..... **1**

1.1 BACKGROUND ..... 1
1.2 SOURCES ..... 3
1.3 ENVIRONMENTAL FATE ..... 3
1.3.1 Persistence ..... 5
1.3.2 Bioaccumulation ..... 6
1.4 TOXICITY ..... 6
1.5 DEFINITION OF RISK ASSESSMENT ..... 7
1.6 PURPOSE AND SCOPE OF RISK ASSESSMENT ..... 7
1.7 APPLICATION OF TOXIC EQUIVALENCE METHODOLOGY ..... 9
1.8 SUMMARY ..... 13

**2 HAZARD ASSESSMENT** ..... **14**

2.1 INTRODUCTION ..... 14
2.2 TOXICITY TO AQUATIC ORGANISMS ..... 15
2.2.1 Freshwater fish ..... 15
2.2.2 Epidemiological studies with wild fish ..... 26
2.2.3 Saltwater fish ..... 27
2.2.4 Freshwater algae and plants ..... 28
2.2.5 Freshwater and marine invertebrates ..... 28
2.2.6 Amphibians and reptiles ..... 29
2.3 SUMMARY OF AQUATIC TOXICITY ..... 31
2.4 AVIAN TOXICITY ..... 32
2.4.1 Laboratory studies ..... 32
2.4.2 Epidemiology studies with wild birds ..... 37
2.4.3 Summary of avian toxicity ..... 48
2.5 MAMMALIAN TOXICITY ..... 48
2.5.1 Acute and chronic toxicity ..... 49
2.5.2 Reproductive toxicity ..... 49
2.5.3 Toxicity studies with mink ..... 50
2.5.4 Epidemiological studies with wild mammals ..... 56
2.5.5 Semi-field studies with marine mammals ..... 57
2.5.6 Summary of mammalian toxicity ..... 59
2.6 CONCLUSION ..... 59

**3 EXPOSURE ASSESSMENT** ..... **61**

3.1 INTRODUCTION ..... 61
3.2 METHODS AND APPROACH ..... 63
3.2.1 Defining bioaccumulation ..... 63
3.2.2 Determination of BSAFs ..... 64
3.3 TERRESTRIAL EXPOSURE ..... 65
3.3.1 Soils ..... 65
3.3.2 Terrestrial mammals ..... 67
3.3.4 Avian wildlife ..... 72

3.4 BIOTA-SOIL ACCUMULATION FACTORS FOR TERRESTRIAL FAUNA ..... 75
3.4.1 BSAFs for marsupials ..... 77
3.4.2 BSAFs for monotremes ..... 77
3.4.3 BSAFs for birds ..... 78
3.5 AQUATIC EXPOSURE ..... 80
3.5.1 Sediment ..... 81
3.5.2 Bivalves and fish ..... 82
3.5.3 Marine mammals ..... 85
3.6 BIOTA-SEDIMENT ACCUMULATION FACTORS FOR AQUATIC FAUNA ..... 87
3.6.1 BSAFs for bivalves ..... 87
3.6.2 BSAFs for fish ..... 88
3.6.3 BSAFs for marine mammals ..... 90
3.7 COMPARISON WITH OTHER STUDIES ..... 90
3.7.1 Bioaccumulation ..... 90
3.7.2 Maternal transfer studies ..... 93
3.8 SUMMARY AND CONCLUSIONS ..... 96

**4 RISK CHARACTERISATION ..... 98**

4.1 INTRODUCTION ..... 98
4.2 RISK CHARACTERISATION METHODS ..... 99
4.2.1 Risk quotients ..... 99
4.2.2 Probability distribution curves ..... 100
4.2.3 Expressing exposure and effects in comparable units ..... 100
4.3 RISK TO BIVALVES ..... 103
4.4 RISK TO FISH ..... 104
4.4.1 Most sensitive TRV for fish ..... 104
4.4.2 Exposure reference values in fish ..... 104
4.4.3 Predicted exposure from sediment levels ..... 106
4.5 RISK TO BIRDS ..... 108
4.5.1 Toxicity reference values ..... 108
4.5.2 Exposure reference values ..... 111
4.5.3 Species sensitivity distributions ..... 114
4.5.4 Exposure to bird embryos based on soil concentrations ..... 116
4.6 RISK TO MAMMALS ..... 119
4.6.1 Aquatic mammals ..... 119
4.6.2 Predicted exposure based on sediment concentrations ..... 121
4.6.2 Terrestrial mammals ..... 122
4.7 UNCERTAINTY ANALYSIS ..... 125
4.7.1 Uncertainties in the conceptual model ..... 126
4.7.2 Uncertainties associated with effects ..... 126
4.7.3 Uncertainties associated with exposure ..... 127
4.8 SUMMARY AND CONCLUSIONS ..... 129

**5. REFERENCES ..... 132**

# Figures


Figure 1.1 A conceptual model of how TEQ methodology is applied to risk assessment ..... 12
Figure 3.1 BSAFs of PCDDs (1-7), PCDFs (8-17) and PCBs (18-29) for various marsupial species ... 77
Figure 3.2 BSAFs of PCDDs (1-7), PCDFs (8-17) and PCBs (18-29) for monotremes ..... 78
Figure 3.3 BSAFs for birds from different trophic levels and regions compared to soils within those regions ..... 79
Figure 3.4 BSAFs of PCDDs (1-7), PCDFs (8-17) and PCBs (18-29) for different species of raptors from South Australia compared to South Australian soils ..... 80
Figure 3.5 BSAFs of PCDDs (1-7), PCDFs (8-17) and PCBs (18-29) for bivalves ..... 89
Figure 3.6 BSAFs of PCDDs (1-7), PCDFs (8-17) and PCBs (18-29) for fish ..... 89
Figure 3.7 BSAFs of PCDDs (1-7), PCDFs (8-17) and PCBs (18-29) for marine mammals (n = 13) compared to marine sediments (n=12) ..... 90
Figure 4.1 Species sensitivity and exposure distribution curves of TEQs in fish eggs ..... 107
Figure 4.2 Species sensitivity distribution and TEQs in bird eggs ..... 115
Figure 4.3 Species sensitivity distribution and TEQs in raptor eggs calculated from tissue levels and predicted from BSAFs ..... 117
Figure 4.4 Predicted TEQs in aquatic mammals, using average BSAFs, compared to the range of threshold TRVs (vertical dashed lines) ..... 122


# Tables


Table 1.1 Dioxin, furan, and PCB congeners with dioxin-like toxicity and properties ..... 2
Table 1.2 Physical and chemical properties ..... 4
Table 1.3 $WHO_{98}$ TEFs for mammals, birds, and fish ..... 10
Table 2.1 Summary of toxic effects on fish exposed to TCDD in water ..... 17
Table 2.2 Summary of toxic effects on fish exposed to TCDD in food ..... 20
Table 2.3 Body burden in female brook trout and corresponding egg burden ..... 20
Table 2.4 Summary of toxic effects on fish exposed to TCDD by single injection ..... 24
Table 2.5 TCDD developmental toxicity in freshwater fish species ..... 25
Table 2.6 Developmental toxicity in bird embryos following a single egg injection ..... 35
Table 2.7 Summary of studies with wild birds examining exposure to TCDD or other dioxin-like chemicals ..... 38
Table 2.8 Comparative toxicity in mammals following dietary exposure to TCDD ..... 49
Table 2.9 TCDD acute developmental and reproductive toxicity in mammals following a single dose exposure ..... 52
Table 2.10 TCDD chronic reproductive and developmental toxicity in mammals following multiple dose exposure ..... 53
Table 2.11 Toxicity endpoints in mink based on tissue concentrations ..... 55
Table 2.12 NOAELs and LOAELs for total PCBs and TEQs in seals ..... 58
Table 2.13 Summary of available data and toxicity of TCDD ..... 60
Table 3.1 Total PCDD, PCDF and PCBs, expressed as TEQ, in Australian soils ..... 66
Table 3.2 TEQs in macropods ..... 69
Table 3.3 TEQs in other marsupials ..... 70
Table 3.4 TEQs in monotremes ..... 70
Table 3.5 TEQs in dingo and a goanna ..... 72
Table 3.6 TEQs in avian wildlife calculated from the full LOD ..... 73
Table 3.7 Body weight, diet, movement and preferred habitat of avian species sampled in the NDP survey ..... 74
Table 3.8 Total PCDD, PCDF and PCBs, expressed as TEQs, in Australian sediments ..... 81
Table 3.9 Total PCDD, PCDF and PCBs, expressed as TEQs, in bivalves from freshwater (F), estuarine (E) and marine (M) environments ..... 82

Table 3.10 Total PCDD, PCDF and PCBs, expressed as TEQs, in fish from freshwater, estuarine, and marine locations ..... 84
Table 3.11 TEQs in aquatic mammals ..... 86
Table 3.12 PCBs (ng/g lipid) and BSAFs in tidal flat and coastal organisms of different trophic levels in Japan ..... 92
Table 3.13 BMFs and BSAFs at different trophic levels in the Great Lakes ecosystem ..... 92
Table 3.14 Mean egg-whole tissue ratios for adult herring gulls from Lake Ontario (Braune and Norstrom, 1989) ..... 93
Table 3.15 Mean BMFs (wet weight basis) for selected congeners between adult herring gulls and fish (alewife) from Lake Ontario ..... 94
Table 3.16 Transfer ratios for PCDD/Fs from soil to eggs ..... 95
Table 4.1 Estimated TEQs in fish eggs and resulting risk quotients (Q) ..... 105
Table 4.2 TRVs derived from field and laboratory studies with birds exposed to dioxin-like compounds ..... 108
Table 4.3 Estimated TEQs in the bird eggs following maternal transfer ..... 112
Table 4.4 Risk quotients derived from predicted TEQs in bird eggs, and using the NOAEL for the most sensitive and most similar species ..... 113
Table 4.5 TRVs used in risk assessments of avian wildlife ..... 114
Table 4.6 Geometric mean NOAELs for embryo mortality and developmental impairment in birds exposed to dioxins in laboratory studies ..... 115
Table 4.7 PCBs (ng/g lipid), TEQs and resulting risk quotients in aquatic mammals ..... 120
Table 4.8 NOAELs and LOAELs for developmental impairment in rats following exposure to TCDD ..... 123
Table 4.9 A summary of toxicity endpoints in mink based on tissue concentrations ..... 124
Table 4.10 TEQs and Q values in terrestrial mammals, using a TRV of 167 pg/g lipid ..... 124

# 1 Introduction

## 1.1 Background

Polychlorinated dibenzo-p-dioxins (PCDD), polychlorinated dibenzofurans (PCDF) and coplanar polychlorinated biphenyls (PCB), collectively referred to as "dioxins" in this report, are among a group of twelve persistent organic pollutants, which were identified in the Stockholm Convention Treaty on Persistent Organic Pollutants (POPs) (2001) as priority substances for elimination or restriction of release worldwide. The concerns surrounding these chemicals include their high toxicity, resistance to biological and chemical breakdown, and their ability to bioaccumulate in organisms. The 2001 Stockholm Convention on Persistent Organic Pollutants (POPs) entered into force on May 17, 2004, marking the start of international efforts to rid the world of dioxins, furans, PCBs and nine pesticides.

Dioxins enter the environment primarily through atmospheric release when they form as unintentional by-products of combustion (both natural and anthropogenic), and during certain types of chemical manufacturing and industrial processes. Once released, dioxins have the ability to undergo long-range transport in the atmosphere, and consequently are widespread environmental contaminants, occurring even at remote locations away from point sources of release. Until recently, data on dioxin levels in Australia suggested that, apart from a few sites with local contamination, Australian dioxin levels are low in comparison to other regions. However, because of the limited amount of measured data, definite conclusions on ambient environmental levels, and the impacts of these levels on humans and wildlife could not be determined.

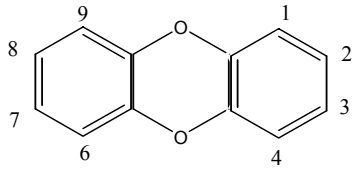
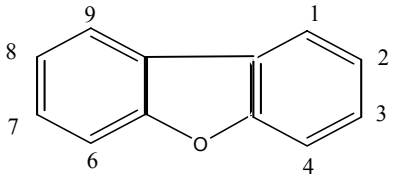
To address the data gaps and concerns surrounding the presence of dioxins in Australia, the Australian Government provided funding over four years for a National Dioxins Program[1] (NDP). This program was initiated in three phases. Phase one involved determining the ambient levels of dioxins in a range of environmental media in Australia. Phase Two involved conducting a human health and ecological risk assessment for Australian native wildlife based on the measured environmental levels. Phase three will involve determining appropriate measures to reduce these chemicals in Australia. This document outlines the findings of the ecological risk assessment addressed in phase two of the NDP. The outcomes of the risk assessment will be used to inform phase three of the NDP involving the development of appropriate measures to reduce or eliminate dioxins in Australia.

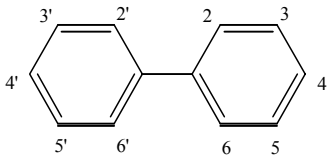
The risk assessment is primarily concerned with PCDD, PCDF and coplanar PCB congeners with "dioxin-like" toxicity and properties. The term "dioxin-like" refers to those congeners that share structural relationships and a common mechanism of toxic action. Of the 419 possible PCDD (dioxins), PCDF (furans) and PCB congeners, 29 are currently considered dioxin-like (WHO, 2001). These are shown in Table 1.1. For simplicity, in this

[1] http://www.ea.gov.au/industry/chemicals/dioxins/index.html

document, the 29 dioxin-like congeners will be referred to as “dioxins”, unless a distinction needs to be made between individual congeners or classes of congeners (i.e. dioxins, furans and PCBs).

**Table 1.1 Dioxin, furan, and PCB congeners with dioxin-like toxicity and properties**

| Congeners | General structure |
|---|---|
| **Dioxins**<br>2,3,7,8–tetraCDD<br>1,2,3,7,8–pentaCDD<br>1,2,3,4,7,8–hexaCDD<br>1,2,3,6,7,8–hexaCDD<br>1,2,3,7,8,9–hexaCDD<br>1,2,3,4,6,7,8–heptaCDD<br>OctaCDD |  |
| **Furans**<br>2,3,7,8–tetraCDF<br>1,2,3,7,8–pentaCDF<br>2,3,4,7,8–pentaCDF<br>1,2,3,4,7,8–hexaCDF<br>1,2,3,6,7,8–hexaCDF<br>1,2,3,7,8,9–hexaCDF<br>2,3,4,6,7,8–hexaCDF<br>1,2,3,4,6,7,8–heptaCDF<br>1,2,3,4,7,8,9–heptaCDF<br>OctaCDF |  |

| PCB Congeners and IUPAC No. | General structure |
|---|---|
| **Non-ortho PCBs**<br>3,3',4,4'–tetrachlorobiphenyl PCB#77<br>3,4,4',5–tetrachlorobiphenyl PCB#81<br>3,3',4,4',5–pentachlorobiphenyl PCB#126<br>3,3',4,4',5,5'–hexachlorobiphenyl PCB#169 |  |
| **Mono-ortho PCBs**<br>2,3,3',4,4'–pentachlorobiphenyl PCB#105<br>2,3,4,4',5–pentachlorobiphenyl PCB#114<br>2,3',4,4',5–pentachlorobiphenyl PCB#118<br>2',3,4,4',5–pentachlorobiphenyl PCB#123<br>2,3,3',4,4',5–hexachlorobiphenyl PCB#156<br>2,3,3',4,4',5'–hexachlorobiphenyl PCB#157<br>2,3',4,4',5,5'–hexachlorobiphenyl PCB#167<br>2,3,3',4,4',5,5'–heptachlorobiphenyl PCB#189 | |

## 1.2 Sources

Dioxins and furans are unintentional by-products of combustion (both naturally occurring such as volcanos or wildfires and anthropogenic such as waste or fuel incineration and operation of the internal combustion engine) and of certain types of chemical manufacturing and industrial processes[2]. Their presence in the environment is primarily the result of atmospheric release during combustion when small amounts are produced during burning of materials containing precursor chemicals, such as chlorinated hydrocarbons. Dioxins and furans may also be produced as contaminants during the manufacture of commercial industrial and agricultural chemicals containing chlorinated phenols (e.g. wood preservatives, pesticides, bacteriocides, tanning agents), or during combustion of materials treated with chlorophenols. Certain industrial processes, such as elemental chlorine bleaching of pulp and paper are additional sources of dioxins and furans. Dioxins and furans may also be present in the environment as a result of historical contamination.

PCBs were once produced commercially for use in a range of industrial and commercial applications because of their low flammability, chemical stability, high boiling point and electrical insulating properties. These applications included; use in electrical, heat transfer, and hydraulic equipment; as plasticisers in paints, plastics and rubber products; and in pigments, dyes and carbonless copy paper. Production of PCBs was ceased in developed countries in the 1970s, and while they are no longer produced commercially, they may be present in the environment as a result of historical contamination, or in older electrical equipment and other products from where they could enter the environment. To prevent release of PCBs, a PCB Management Plan is in place, under the National Strategy for the Management of Scheduled Wastes[3]. Dioxin-like PCBs may also form unintentionally as by-products of combustion.

## 1.3 Environmental fate

The physical and chemical properties of chemical substances are good predictors of how the chemical will behave and partition in the environment (Table 1.2). For example, the vapour pressure and Henry's Law Constant are measures of a chemical's volatility, and hence, its atmospheric fate. The octanol-water partition coefficient ($K_{ow}$) is a measure of a chemical's solubility in lipid and, hence, is a good indicator of bioaccumulation potential. Chemicals with high log $K_{ow}$ (>5) have low water solubility and a high potential to bioaccumulate in the fatty tissue of organisms. The bioaccumulation factor (BAF) and bioconcentration factor (BCF) are measures of this bioaccumulation.

Dioxins, furans and PCBs are semi-volatile and can be transported in either gaseous form or attached to dust and ash particles. The relative proportion occurring in the gaseous or particulate phase depends on an individual congener's chemical properties (vapour

[2] Australia has initiated a dioxin inventory of sources of emissions of polychlorinated dioxins and furans to air (http://www.npi.gov.au)

[3] http://www.deh.gov.au/industry/chemicals/scheduled-waste/pcbs/plan/note.html

pressure, number of chlorine atoms etc.), and on the air temperature. In general, as the number of chlorine atoms increases, the temperature at which half of the dioxin will be present in the gaseous form increases. Dioxins with more than five chlorine atoms tend to partition mainly into particulate phases.

**Table 1.2 Physical and chemical properties**

| Property | PCDD[1] | PCDF[1] | PCB[2] |
|---|---|---|---|
| Octanol/water partition coefficient (log Kow) | 6 - 9 | 6 - 9 | 4.46 - 8.18 |
| Water solubility (µg/L) | 0.019 | 0.692 | 2.7 - 590 |
| Vapour pressure (mm Hg) | $1.5 \times 10^{-9}$ to $3.4 \times 10^{-5}$ | $9.21 \times 10^{-7}$ | $7.7 \times 10^{-5}$ to $4.1 \times 10^{-3}$ |
| Henry's law constant (atm $m^3$/mol) | $1.6 \times 10^{-5}$ to $1.0 \times 10^{-4}$ | $1.48 \times 10^{-5}$ | $5.2 \times 10^{-4}$ to $2.0 \times 10^{-3}$ |
| BAF or BCF | 130,000 | 61,000 | 60,000 - 270,000[3] |

[1]Opperhuizen, (1991); US EPA (2002),
[2]WHO (1993) for selected Aroclors; water solubility, vapour pressure and Henry's law measured at 25º C, [3]PCB BCF in fish for selected Aroclors.

Wania and Mackay (1996) have hypothesized a global fractionation process for the long-range transport of persistent organic pollutants, including dioxins, where POPs released in one part of the world are transported by a process of repeated evaporation and deposition to other regions, such that the less volatile (and most persistent) PCDD/PCDFs accumulate in warmer tropical and sub-tropical environments, while the lower chlorinated PCDD/PCDFs accumulate in colder climates and in the polar regions.

The most important pathway for removal of dioxins and furans from the atmosphere, and the main route through which dioxins enter aquatic and terrestrial environments, is by gravitational settling and washout in rain. Dioxins attached to particulate matter will tend to settle out under gravity, with the coarser particles deposited more rapidly and closer to emission sources than those attached to fine particles. Both fine particulate dioxins and dioxins in gaseous form are more amenable to long-range atmospheric transport by wind. These particles will tend to be deposited when washed out of the atmosphere by rain and snow, although they may have traveled far from the emissions source before they are eventually removed.

Dioxins deposited in aquatic environments will adsorb strongly to dissolved or particulate organic carbon suspended in the water column, particularly in estuarine and near shore marine environments, where they may be present as an ongoing source of contamination. Dioxins adsorbed to particulate matter tend to rapidly redistribute (half-life time <4 days) by settling out to bottom sediments (Currie et al., 2000). The strength of adsorption to sediment generally increases with increasing numbers of chlorine atoms. Dioxins deposited on the ground surface in terrestrial environments adsorb to organic matter in the soil and suspended in pore water. Dioxins adsorbed to soil particles may subsequently enter the aquatic environment via runoff and soil erosion. Dioxins are not expected to leach from the

soil to groundwater owing to their low water solubility and their strong adsorption to particulate organic matter.

### 1.3.1 Persistence

Environmental persistence is a key characteristic of dioxins and is a major reason why they become widespread environmental contaminants following their release. A chemical is considered persistent if it is resistant to biological and chemical breakdown in the atmospheric, aquatic, or terrestrial environment. Atmospheric degradation of chemicals occurs primarily through reactions with hydroxyl (OH) radicals, and photolysis (light-induced degradation), while primary mechanisms for degradation of chemicals in soil, sediment and water are microbial (i.e. by bacteria and other microorganisms) and chemical (hydrolysis, photolysis, and other chemical reactions).

Degradation rates of chemicals in general vary with environmental conditions, such as temperature, moisture content, microbial biomass, and the amount of sunlight available, and with a particular degradation mechanism or pathway. Consequently, the persistence of dioxins will vary according to the environmental media (soil, sediment, water, air) and the climatic conditions under which they occur. The persistence of individual dioxin-like congeners also varies according to their chemical structure.

Persistence is most commonly measured in terms of the half-life time ($t_{½}$), which describes the time in takes (in hours, days, months, or years) for half of the chemical to be degraded. Chemicals are classified as persistent if their half-life times in air are >two days, in water are >two months, and in soil and sediment >six months (Stockholm Convention, 2001).

Dioxins have relatively long atmospheric half-life times, which enables them to be transported for long distances in the atmosphere. In soil and sediment, dioxins bind strongly to organic matter and tend to also persist for long periods. One might expect dioxin residues in surface soil to be more sensitive to fluctuations in concentration than residues in buried soil or in aquatic sediments owing to the seasonal fluctuations in soil organic matter. Turnover of soil organic matter would be expected to release some of the adsorbed dioxins exposing them to microbial degradation and volatilisation. The higher chlorinated congeners are the most resistant to breakdown and volatilisation in soil (Orazio et al., 1992). Estimates of the half-life of 2,3,7,8-TCDD on the soil surface range from 9 to 15 years, whereas the half-life in subsurface soil may range from 25 to 100 years (Bacci et al., 1990).

In many regions, the presence of dioxin, furans and PCBs in aquatic sediments and soils typically reflect historical, rather than current emissions. For persistent organic pollutants, environmental levels can build up over decades and may not reach peak levels for many decades, even though emissions remain constant. Conversely, when emissions are reduced, levels will decrease gradually until a new steady state is reached, reflecting equilibrium with the lower reduced levels of emissions.

### 1.3.2 Bioaccumulation

Dioxins generally have low solubility in water and high solubility in lipids. Dioxins and furans have very high log $K_{ow}$ values between 6 to 9 (Table 1.2). PCBs (all congeners) have log $K_{ow}$ values between 4.46 and 8.18 (Hawker and Connell, 1988), with the tetra- and higher chlorinated (dioxin-like) PCBs having the highest values.

Because of their high affinity to lipids, dioxins can accumulate in the bodies of organisms when they are exposed to these chemicals in water, soil, sediment, or in their diet. Dietary intake is the primary mechanism governing the levels of dioxins and dioxin-like chemicals in biota (Gobas et al., 1993), although ultimately, the levels in the diet depend on the levels in soil and sediment.

Dioxins in soil or sediment are adsorbed by plants or taken up by organisms through direct consumption of soil or sediment. When these organisms and plants are, in turn, eaten, the dioxins stored in them are then taken up by the organisms that consumed them. Because dioxins are persistent and not broken down and excreted by normal metabolic processes in animals, the chemicals move into the animal's lipids for storage. In this way, dioxins are transferred from one organism to another when predators consume their prey. Dioxins may increase in concentration at each successive trophic level because a predator needs to eat numerous prey organisms, and in doing so, takes up into storage most of the dioxins present in all the individual prey organisms. Therefore, the receptor organisms most at risk from exposure to dioxins are those occupying the top of the food chain (Jones et al., 1993).

## 1.4 Toxicity

Studies with laboratory animals show that exposure to dioxins results in a wide spectrum of toxic responses in a range of vertebrate species. Depending on the duration of exposure and the dose, these toxic responses range from cellular level biochemical effects to acute lethality. Reported adverse responses include:

- acute lethality
- reproductive impairment
- developmental abnormalities in young
- endocrine and immune dysfunction
- neurological dysfunction
- wasting syndrome
- edema and hemorrhaging.

Not all dioxin-like congeners are equally toxic, however, and not all of the effects caused by exposure are observed in every single species, but rather, the adverse effects can differ between species, life-stage, and in some cases, even the gender of test animals.

Dioxin-like PCDDs, PCDFs and PCBs share a common toxic mechanism, which involves binding to a cellular protein, known as the aryl hydrocarbon (Ah) receptor, where they form complexes that translocate to the nucleus and initiate changes in gene expression. Binding to this receptor appears to be the initial step leading to biochemical, cellular, and tissue-level changes occurring in organisms exposed to dioxins (Hahn and Stegeman, 1992). Whether individual PCDD, PCDF and PCB congeners have dioxin-like toxicity or not, depends largely on their chemical structure, namely the number and position of the chlorine atoms on the dioxin molecule. These two factors determine the shape of the molecule and the ability of a particular congener to fit to the Ah receptor (US EPA, 2000d).

The presence of an Ah receptor in an organism also governs whether dioxins are toxic to the organism or not. The observed lack of sensitivity of plants and invertebrates is consistent with the view that the Ah receptor is not present in invertebrates.

## 1.5 Definition of risk assessment

In general terms, risk assessment of chemicals is defined as the process of estimating the potential impact of a chemical, or a group of chemicals, on a specified human population or ecological system given a specific set of conditions and time frame. Ecological risk assessment (ERA) of chemicals is specifically concerned with determining the potential impact of chemicals to non-human organisms in the environment.

The risk assessment process can be divided in four distinct phases. These phases are: (1) issue identification; (2) hazard assessment; (3) exposure assessment and (4) risk characterisation. The issues identification phase of risk assessment provides the background and context for the risk assessment, defines the purpose, goals and scope of the risk assessment, and outlines the approach used to conduct the risk assessment.

The hazard assessment (also referred to as the effects assessment or the dose-response analyses) examines the adverse toxic effects observed in organisms when exposed to chemicals, and determines the relationship between the level of exposure and the incidence and severity of the toxic effects. The exposure assessment estimates the chemical concentration in each environmental compartment to which organisms may be exposed, determines the main pathways of exposure, and identifies the receptor species potentially exposed. For all chemicals, risk is a function of exposure and toxicity. As such, the risk characterisation phase of risk assessment determines the likelihood and severity of risk to wildlife posed by exposure to chemicals through the integration of information from the hazard and the exposure assessments.

## 1.6 Purpose and scope of risk assessment

The main aims of the ERA are to determine whether there is a potential risk to native fauna exposed to dioxins at the ambient levels currently occurring in the Australian environment. The broad objectives of the ERA are summarised as follows:

- Assess the level of exposure to Australian fauna on the basis of trophic level in key aquatic and terrestrial environments

- Compare the levels of dioxins in key aquatic and terrestrial environments with the level known to cause toxic effects in organisms
- Identify the groups or populations of organisms potentially at risk from the adverse effects of exposure to dioxins and determine any differences in trends between regions, land-uses, and trophic level.

No ecotoxicology studies were performed specifically for this risk assessment. The hazard (effects) assessment will rely on ecotoxicology studies in published scientific journals, existing reports and databases for the dose-response analysis. The exposure assessment will rely predominantly on data collected during the national data-gathering survey, which was initiated to determine ambient dioxin levels in range of Australian environmental media including air, aquatic sediments and soil. Levels in the tissue of fauna were alos determined. The NDP surveys are the subject of separate reports, with the following titles:

1) Determination of Ambient Levels of Dioxins in Australia - Ambient Air, Draft Final Report (Gras and Müller, 2003)
2) Dioxins in Soils in Australia, Draft Final Report (Müller et al., 2003a)
3) Dioxins in Aquatic Environments in Australia, Draft Final Report (Mueller et al., 2003b)
4) Dioxins in Fauna in Australia, Draft Final Report (Correll et al., 2004).

Because inhalation is not expected to be a major exposure route for native fauna, the exposure assessment will use data only from surveys 2, 3, and 4 (above) to assess exposure. The reports are referred to in subsequent chapters as NDP soil, aquatic sediment, and fauna surveys, respectively.

The NDP surveys determined dioxin levels in sediment and soil at sites in airsheds throughout Australia within the general categories of estuarine, marine and freshwater aquatic environments, and agricultural, remote, urban and industrial terrestrial environments. The strategy employed in both surveys was to sample sites not impacted by immediate point sources and, hence, determine baseline dioxin loads.

The NDP fauna survey determined dioxin levels in a limited number of aquatic and terrestrial fauna species from different trophic levels gathered from locations around Australia within broad site categories of southeastern, southwestern, and northern. The strategy adopted in the fauna survey was to sample recently deceased animals, opportunistically, most commonly from road kills. No animals were intentionally sacrificed for determination of dioxin loads. These species are classified in this ERA under four broad classes of organisms: aquatic invertebrates (bivalves), birds, fish and mammals.

Because of the opportunistic sampling strategy adopted in the fauna survey, and the broad, national focus of the data gathering surveys, the risk assessment cannot be definitive, but rather will provide a preliminary indication of the potential risk dioxins pose to Australian ecosystems at current baseline levels in the environment.

## 1.7 Application of toxic equivalence methodology

Dioxins exist in the environment as complex mixtures rather than as single chemical compounds, with the potency of each individual dioxin-like congener in a mixture differing considerably. These differences must be taken into account when determining the toxicity of environmental mixtures of dioxins. For this purpose, the concept of Toxicity Equivalents has been developed, whereby the toxicity of a mixture is expressed in terms of a single value, or TCDD Toxicity Equivalence Concentration (TEQ), which reflects the combined toxicity of all dioxins in the mixture.

The TCDD TEQ (henceforth called TEQ) in an environmental sample (i.e. sediment, soil, organism) is calculated by analytically determining the levels of each congener in the sample ($C_n$), multiplying the concentration of each congener by a congener-specific Toxicity Equivalency Factor (TEF), and then adding all products to give a TEQ (Van den Berg et al. 1998). The TEQ represents the amount of TCDD required to equal the combined effects of all dioxins in a mixture (USEPA, 2000b).

$$TEQ = (C_1 \times TEF_1) + (C_n \times TEF_n) + \ldots + (C_{29} \times TEF_{29})$$

The TEF describes the potency of each dioxin-like congener relative to 2,3,7,8-TCDD (henceforth called TCDD), which is the most potent of all of the congeners. TCDD is assigned a TEF of one, with all other congeners assigned an order of magnitude toxicity ranking relative to TCDD. A number of different TEF systems have been developed since the method was first applied in the 1980s. The most recent system is the $WHO_{98}$ TEF system, which was developed through international scientific consensus by a panel of experts at a WHO meeting using all available toxicity data (Van den Berg et al., 1998). The WHO TEF system will be adopted in this ERA. In the $WHO_{98}$ TEF system, separate TEFs have been developed for birds, fish, and mammals, owing to differences in the sensitivity to certain congeners between the classes of organisms (Table 1.3).

Fish-specific TEFs have been derived from experimental studies of mortality in early life-stage rainbow trout following exposure to different dioxin-like congeners, in addition to *in vivo* and *in vitro* studies for cytochrome[4] (CYP1A) induction. Bird-specific TEFs are derived from egg injection studies, and *in vitro* studies (i.e. with cultured avian hepatocytes and thymus cells), for a number of bird species. Mammalian TEFs are based largely on rodent studies exposed to dioxins in their diet. To date, not enough information is available to determine TEFs specific to amphibians and reptiles (Van den Berg et al. 1998).

The validity of the "toxicity equivalence" method depends on two important assumptions. The first is that the individual dioxin-like congeners act via the same mechanism to cause toxicity, and the second is that these toxic effects are additive.

A conceptual model of how toxic equivalence methodology is applied within the context of the current risk assessment framework is shown in Figure 1.1. In the hazard assessment, toxicity endpoints derived from studies with dioxins, are expressed in terms of the tissue concentrations (i.e. the body burden), which resulted in a given effect level, such as $LD_{50}$

[4] Enzymes involved in metabolism in cells.

(see glossary). If the toxicity test was conducted with fish using PCB 126, the resulting toxicity endpoint will be multiplied by the congener- and fish-specific TEF, in this case 0.005 (Table 1.3) to get the TEQ, whereas if the test species was a bird, the resulting toxicity endpoint will be multiplied by the bird-specific TEF of 0.1 to get the TEQ.

Similarly, in the exposure assessment, the concentrations of each dioxin-like congener found in the tissue of a fauna sample are converted to a single TEQ (or exposure reference value) by way of the congener and species-specific TEFs. If the sample is a bird, all congener concentrations will be multiplied by the congener and bird-specific TEFs, whereas if the sample is a fish, the congener concentrations will be multiplied by congener and fish-specific TEFs. The exposure reference value TEQ is then compared to a toxicity reference value TEQ to determine the risk to the organism.

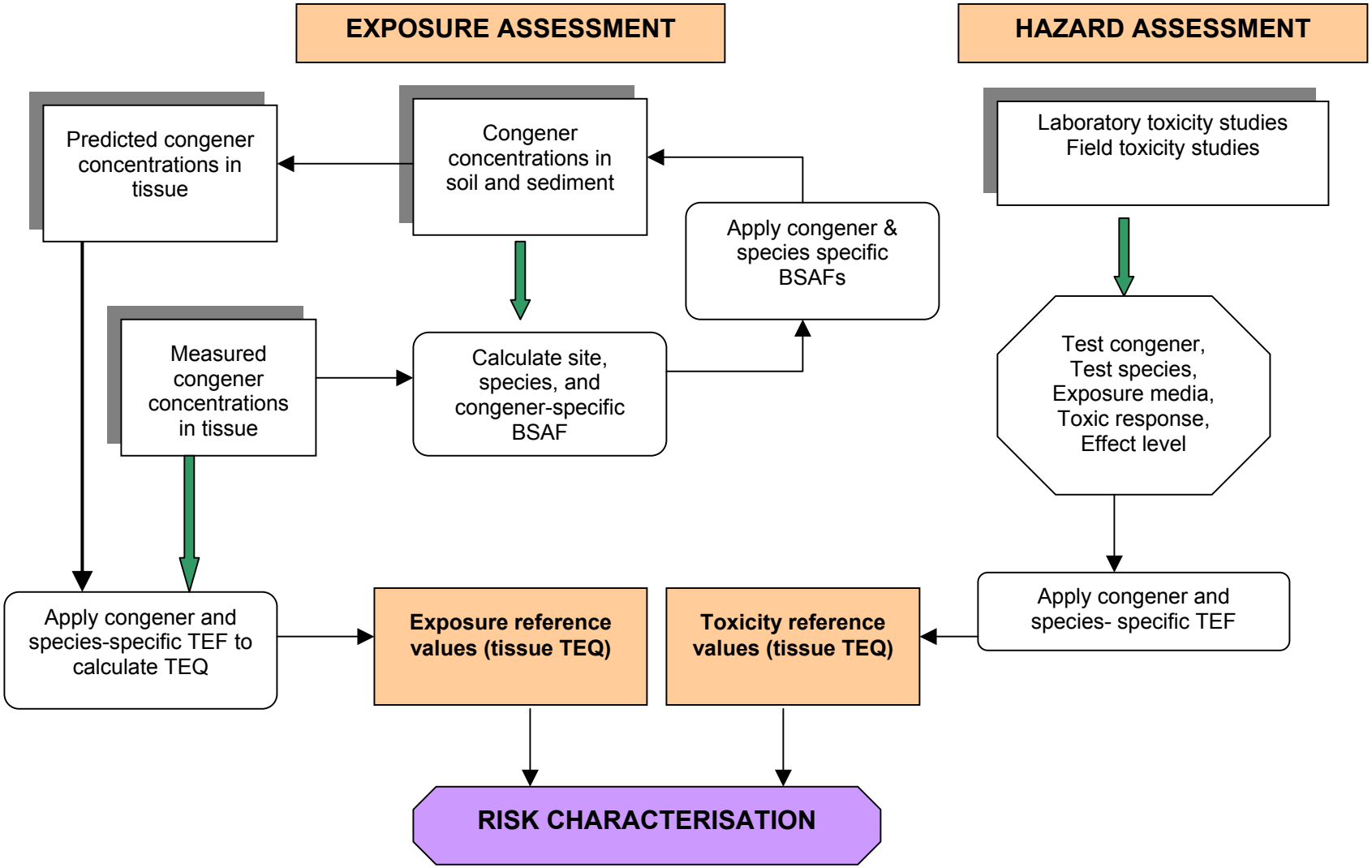
**Table 1.3 $WHO_{98}$ TEFs for mammals, birds, and fish[5]**

| Congeners | Mammals | Birds | Fish |
|---|---|---|---|
| **Dioxins** | | | |
| 2,3,7,8–tetraCDD | 1 | 1 | 1 |
| 1,2,3,7,8–pentaCDD | 1 | 1 | 1 |
| 1,2,3,4,7,8–hexaCDD | 0.1 | 0.05 | 0.5 |
| 1,2,3,6,7,8–hexaCDD | 0.1 | 0.01 | 0.01 |
| 1,2,3,7,8,9–hexaCDD | 0.1 | 0.1 | 0.01 |
| 1,2,3,4,6,7,8–heptaCDD | 0.01 | <0.001 | 0.001 |
| OctaCDD | 0.0001 | 0.0001 | <0.0001 |
| **Furans** | | | |
| 2,3,7,8–tetraCDF | 0.1 | 1 | 0.05 |
| 1,2,3,7,8–pentaCDF | 0.05 | 0.1 | 0.05 |
| 2,3,4,7,8–pentaCDF | 0.5 | 1 | 0.5 |
| 1,2,3,4,7,8–hexaCDF | 0.1 | 0.1 | 0.1 |
| 1,2,3,6,7,8–hexaCDF | 0.1 | 0.1 | 0.1 |
| 1,2,3,7,8,9–hexaCDF | 0.1 | 0.1 | 0.1 |
| 2,3,4,6,7,8–hexaCDF | 0.1 | 0.1 | 0.1 |
| 1,2,3,4,6,7,8–heptaCDF | 0.01 | 0.01 | 0.01 |
| 1,2,3,4,7,8,9–heptaCDF | 0.01 | 0.01 | 0.01 |
| OctaCDF | 0.0001 | 0.0001 | <0.0001 |
| **PCB Congeners IUPAC #** | | | |
| **Non-ortho PCBs** | | | |
| PCB#77 | 0.0001 | 0.05 | 0.0001 |
| PCB#81 | 0.0001 | 0.1 | 0.0005 |
| PCB#126 | 0.1 | 0.1 | 0.005 |
| PCB#169 | 0.01 | 0.001 | 0.00005 |
| **Mono-ortho PCBs** | | | |
| PCB#105 | 0.0001 | 0.0001 | <0.000005 |
| PCB#114 | 0.0005 | 0.0001 | <0.000005 |
| PCB#118 | 0.0001 | 0.00001 | <0.000005 |

[5] Reference:Van den Berg et al., 1998

| | | | |
|---|---|---|---|
| PCB#123 | 0.0001 | 0.00001 | <0.000005 |
| PCB#156 | 0.0005 | 0.0001 | <0.000005 |
| PCB#157 | 0.0005 | 0.0001 | <0.000005 |
| PCB#167 | 0.00001 | 0.00001 | <0.000005 |
| PCB#189 | 0.0001 | 0.00001 | <0.000005 |

**Figure 1.1** **A conceptual model of how TEQ methodology is applied to risk assessment**



Soil and sediment concentrations can be used to assess exposure, however, point concentrations in soil and sediment cannot be related directly to the toxicity reference values, even if expressed in terms of TEQs, because the pattern of congeners will vary between abiotic media (i.e. soil and sediment) and biotic media (i.e. organisms). These variations result from the differences between individual congeners in bioavailability, uptake and elimination in the digestive tract. Patterns will also vary among different species and at different trophic levels owing to differences in bioaccumulation potential. This will result in the corresponding total TEQ being different in the soil and organism sample. The concentrations of each specific congener in the soil may be higher or lower than the corresponding concentration in the tissue of the exposed organisms.

In order to relate dioxin exposure concentrations in soil and sediment to exposure and effects levels in organisms, soil and sediment concentrations must first be converted to congener concentrations in the tissue of the receptor organism (or tissues of their food items), prior to applying TEFs for calculating TEQs (US EPA, 2003). This is achieved by way of biota-sediment accumulation factors (BSAF) (Figure 1.1). BSAFs are calculated from the ratio of the congener concentration in tissue and the congener concentration in soil or sediment. The BSAFs for one species can then be applied to similar species, for which no data are available, to predict their tissue concentrations from soil or sediment concentrations. This topic is discussed further in the Chapters 3 and 4.

## 1.8 Summary

This ERA aims to establish the potential risk of adverse effects to Australian ecosystems caused by exposure to mixtures of PCDDs, PCDFs and PCBs with dioxin-like toxicity. Toxic Equivalence methodology is adopted to assess the risk from exposure to mixtures of dioxins at ambient environmental loads. The ERA is a screening level risk assessment, with a broad, nationwide focus, intended to provide a preliminary indication of the potential risk dioxins pose to Australian fauna and ecosystems.

The document is divided into 4 chapters, reflecting each of the four elements of risk assessment. The issues identification has been covered in this chapter and is not discussed further. The second chapter comprises the ecotoxicological assessment, which covers the hazard assessment phase of the ERA. The hazard assessment relies on published toxicology data derived from studies with laboratory animals and field studies with wildlife to determine the toxic effects of dioxins. The third chapter comprises the exposure assessment. The exposure assessment will rely predominantly on baseline dioxin levels measured in biotic and abiotic media in the Australian aquatic and terrestrial environments during the various NDP surveys. The last chapter comprises the risk characterisation phase of risk assessment, in which information from the hazard and exposure assessments is integrated to develop an estimation of the incidence, severity, and likelihood of risk to Australian fauna at current levels of exposure.

# 2 Hazard Assessment

## 2.1 Introduction

This chapter reviews the data on the toxic effects of dioxins to non-human organisms. The data are sourced from published scientific journals, on-line databases, and in existing reports[6] on dioxins. The purpose of the review is to assess the relationship between the dose or exposure level and the kind and severity of the toxic responses following exposure to dioxins. Another important aim of the toxicological assessment is to determine appropriate toxicity reference values (TRV) for use in risk characterisation, which can be compared to the environmental levels to which organisms are exposed (Chapter 4). To this end, two types of studies are reviewed, namely, dose-response studies performed under controlled laboratory conditions, and epidemiological studies of wildlife exposed to dioxins in the natural environment.

Controlled laboratory experiments typically fall into two broad categories, those conducted *in vivo* (on whole organisms), and those performed *in vitro* (on cells in test tubes). *In vivo* studies are performed by exposing groups of single species to graded doses of the test substance to determine effect levels at different exposure concentrations. Typical effect levels determined are the $LD_{50}$, the lethal dose at which 50% of test organisms die or are adversely affected, the lowest-observed adverse effects level (LOAEL), and the no-observed-adverse-effects level (NOAEL). *In vitro* studies are performed with cells from liver and other tissue, rather than whole organisms. *In vitro* studies are most often performed to measure biochemical changes. A common measured response is the induction of liver enzymes involved in metabolism of chemicals, including the cytochrome P450-based[7] mixed function oxidase system. Enzyme induction is a useful biomarker indicating an organism has been exposed to dioxins. Biochemical responses are also used to determine the relative potencies of dioxin congeners to cause biochemical changes in cells.

This assessment will focus largely on studies conducted with laboratory animals (*in vivo*) examining the toxic responses in broad classes of organisms, i.e. fish, birds, mammals, and invertebrates. The majority of these studies are performed with 2,3,7,8-tetrachlorodibenzo-

[6] When data are derived from existing reviews, and the original scientific paper has not been consulted, the data are referenced by the name of the review from which they are cited.

[7] The cytochrome P450-based mixed function oxidase (MFO) system has the capability of oxidising a wide range of chemical contaminants. In response to exposure to contaminants, the activity of a number of isoenzymes, such as ethoxyresorufin-*O*-deethylase (EROD) and benzyloxyresofurin-*O*-dealkylase (BROD), increase significantly. The significance of induction is not fully established, however, some research suggests it is an adaptive mechanism to enhance elimination of environmental contaminants (Ahokas *et al*. 1994) and thus, TCDD-induced MFO activities may represent a detoxification process rather than one leading to toxic effects. Other reseach suggests that increases in the metabolism of certain chemicals (e.g. hormones) could lead to adverse effects on biological functioning particulary if it occurs over the long-term or during sensitive life-stages.

p-dioxin (TCDD), which is the most toxic of the dioxin congeners and the reference substance for setting TEFs (Chapter 1). Emphasis is given to those responses most likely to lead to adverse ecological consequences, namely reproductive and developmental impairment following long-term chronic exposure. *In vitro* studies are considered when they provide additional dose-response information on toxicity of dioxins to wildlife species.

Laboratory studies examining the toxic effects of dioxins are available for a limited number of test species and classes of organisms, with relatively few data for wildlife species. Consequently, epidemiological studies are assessed to provide additional information on the toxicology of dioxins in wildlife. Limited epidemiological studies are available relating contaminant levels in wildlife to biological effects. However, unlike laboratory experiments, where variables are controlled and dose-response relationships can be determined; in the natural environment, cause and effect relationships are often difficult to establish because wildlife populations are vulnerable to a variety of chemical and non-chemical stressors.

## 2.2 Toxicity to aquatic organisms

### 2.2.1 Freshwater fish

A large database exists of laboratory studies examining the effects of TCDD on the different life stages of freshwater fish. Much of this data is reviewed in the US EPA (1993a) interim report on TCDD. In these studies, fish are exposed to TCDD through a number of routes including in aquatic media, by injection, and in the diet.

The toxic responses following exposure to TCDD are similar irrespective of the exposure duration or route, although early life stage fish are significantly more sensitive to the toxic effects of dioxins than adult or juvenile fish. Typical responses in early life stage fish include yolk sac edema, hemorrhaging, arrested development, and head and spinal deformities. Responses in older fish include lesions, fin necrosis and death.

A number of characteristic dose-response relationships have been observed in fish following exposure to dioxins. These include:

- Dose dependent response following acute exposure, with higher doses resulting in increased severity of effects
- Time dependent response, with longer exposure periods resulting in increased severity of responses at lower doses
- Delayed mortality following exposure, with the time of death occurring up to several weeks after exposure
- Steep dose-response curves, where the difference between the level causing no effects and those causing lethal effects is quite small.

The observed effects from exposure to TCDD (and other dioxins that bioaccumulate) occur as a function of body burden, rather than the dose level. Dioxins accumulate in tissue until a threshold concentration for a given effect is reached, often resulting in delayed effects

including delayed mortality. The increased severity of effects at higher doses or longer exposure periods results because residues (body burden) in organisms reach the threshold body burden more rapidly with increasing exposure concentration and time.

**Waterborne exposure**

In acute (short-term) studies, rainbow trout (*Oncorhyncus mykiss*) or their eggs are exposed to measured or nominal concentrations of TCDD in water (usually pre-dissolved by a solvent) of between 0.1 ng/L to 107 ng/L for periods from 6 to 96 hours. Following exposure, test organisms are placed in clean water and observed for some time after exposure has ceased. Measured effects range from 100% mortality to sub-lethal effects, such as reduced growth, fin necrosis and delayed hatching of eggs (Table 2.1).

These studies showed that the toxic effects of TCDD do not always become evident during the exposure period, but may begin at significant periods after exposure has ceased, with delayed mortality occurring from weeks to a couple of months after the initial exposure period has ended. For example, in one study, rainbow trout fingerlings exposed to 107 ng/L for six hours and then placed in clean water, initially appeared healthy, but exhibited delayed mortality on days 78, 136, 137 and 139 after exposure.

Similar results are seen when fertilised fish eggs are exposed to TCDD for short periods. The toxic effects manifest in the developing fish some time after hatching. For example, in one study trout eggs exposed to concentrations of 0.10 ng TCDD/L for 96 hours displayed delayed development and reduced fry growth upon hatching for up to 160 days after exposure ceased (US EPA 1993a, references therein). In another study, newly fertilised eggs of brook trout (*Salvelinus fontinalis*) exposed to TCDD in water under static conditions for 48 hours, and then transferred to uncontaminated water and observed through their development, exhibited dose-related toxic effects during and after hatching including sac-fry mortality and associated yolk-sac edema, hemorrhages, and arrested development. The $LD_{50}$ and $LD_{100}$ for brook trout were determined to be 200 (179-215) and 324 (283-488) pg TCDD/g egg, respectively. The NOAEL and LOAEL for sac-fry mortality were 135 and 185 pg TCDD/g egg, respectively (Walker and Peterson, 1994).

The early life stages of fish are significantly more sensitive to TCDD than juvenile and adult fish. For example, 100% mortality occurred when fertilised eggs and sac fry of rainbow trout were exposed to 10 ng/L TCDD for 96 hours. Fingerlings and juveniles required concentrations of 100 ng/L, or 10 times higher, before 100% mortality occurred (US EPA 1993a, and references therein).

Toxicity tests with sac fry show that the effect of higher exposure concentrations is to increase mortality over shorter periods. For example, rainbow trout sac-fry exposed to 1 ng/L TCDD for 96 h exhibited reduced growth and mortality for up to 160 days after exposure, while sac-fry exposed to 10 ng/L for 96 hours had all died by day ten after exposure ceased (US EPA 1993, and references therein).

**Table 2.1 Summary of toxic effects on fish exposed to TCDD in water[8]**

| Species | Concentration (ng/L) | Concentration in organism (pg/g) | Duration Exposure/Observation | Effect/endpoint |
|---|---|---|---|---|
| Coho salmon (*Oncorhyncus kisutch*) juvenile | 0.56<br>5.60 | | 96 h/114 d<br>96 h/56 d | No toxic effect<br>50% mortality (beginning 5-10 d after exposure, and continuing for 2 months). |
| Rainbow trout (*Oncorhyncus mykiss)* eggs | 0.10<br>1.0<br>10<br>1.0 | | 96 h/160 d<br>96 h/160 d<br>96 h/40 d<br>96 h/160 d | Delayed development, reduced fry growth<br>Reduced growth, sac fry mortality<br>100% sac fry mortality<br>Reduced growth, mortality |
| Sacfry | 10<br>12.2<br>1.83<br>0.176 | <br><br><br>3,200 | 96 h/10 d<br>96 h/16 d<br>96 h/21 d<br>28 d/28 d | 100% mortality<br>100% mortality<br>$LC_{50}$<br>95% mortality |
| Swim-up fry | 0.0011<br>0.038<br>0.046 | 21<br>765 | 28 d/28 d<br>28 d/28 d<br>28 d/28 d | NOAEL<br>LOAEL (45% mortality)<br>LC50 |
| Juvenile | 10<br>100<br>100 | | 96 h/68 d<br>96 h/23 d<br>96 h/23 d | Reduced growth, mortality<br>100% mortality<br>100% mortality |
| Fingerling | 107 | 650-2,580 | 6 h/42-139 d | Mortality, fin rot, increased liver weight |
| Northern Pike (*Esox lucius*) eggs | 0.1<br>1.0<br>10 | | 96 h/72 d<br>96 h<br>96 h | Delayed hatch, reduced growth of fry<br>53% mortality to fry<br>99% mortality to fry |
| Fathead minnow (*Pimephales promelas*) juvenile | Water (static)<br>0.049-0.067<br>1.7<br>7.1 | | <br>71 d/61 d<br>28 d<br>24 h/60 d | <br>Mortality and pathology<br>$LC_{50}$<br>40% mortality |
| Channel catfish (*Ictalurus punctatus*) fingerling | Model ecosystem<br>2.4-4.2 | | <br>15-20 d | <br>100% mortality |

[8] From US EPA 1993a, and references therein

**Table 2.1 Continued**

| Species | Test Concentration (ng/L) | Concentration in organism (pg/g) | Duration Exposure/Observation | Effect/endpoint |
|---|---|---|---|---|
| Carp (*Cyprinus carpio*) adult | 0.06 | 2200 | 71 d/61 d | Mortality and pathology |
| Mosquito fish (*Gambusia affinis*) | Model ecosystem<br>2.4-4.2 | | <br>15 d | <br>100% mortality |
| Guppy (*Poecilia reticulate*) juvenile | Water (static)<br>0.1<br>100 | | <br>24 h/42 d<br>120 h/37 d | <br>Significant increase in fin necrosis<br>100% mortality |
| Lake trout (*Salvelinus namaucush*) eggs | Water (renewal) | 34<br>40<br>55<br>65 | 48 h/to post swim up | NOAEL<br>23% mortality in sac fry<br>LOAEL (sac fry mortality)<br>$LC_{50}$ (sac fry) |
| Rainbow trout (*Oncorhyncus mykiss)* eggs | Water (renewal)<br>279<br>439 | | 48 h/to post swim up | <br>Significant mortality in sac fry<br>$LR_{50}$ (sac fry) |
| Japanese medaka (*Oryzias latipes*) eggs | 3.5-6.0<br>9-13<br>14-15<br>14-17 | <br><br><br><br>240 (embryos) | Fertilised egg to 3 days post hatch | $EC_{50}$ (embryos with lesions)<br>$LC_{50}$<br>$EC_{50}$ (embryos with severe lesions)<br>$EC_{50}$ (prevent hatch)<br>$EC_{50}$ in eggs causing 50% effects |

Chronic (longer-term) exposure to TCDD results in higher mortalities at lower concentrations. For example, exposure of the swim up fry of rainbow trout to 0.176 ng/L for 28 days resulted in 95% mortality, compared to lower mortality and reduced growth in the previous study with sac fry exposed to 1 ng/L for 96 h. In other studies, exposure of mosquito fish to 2.4 to 4.2 ng/L TCDD for 15 days resulted in 100% mortality, and exposure of channel catfish fingerlings to the same concentrations for 15-20 days, also resulted in 100% mortality (US EPA 1993a, and references therein).

**Dietary exposure**

The predominant route of dioxin exposure to pelagic fish in the wild is expected to be through the diet, with direct waterborne exposure through the gills likely to be a minor route of uptake owing to the insolubility of dioxins in water. The limited dietary exposure data indicate that the toxic responses from exposure to TCDD in food are similar to those observed for aquatic exposure. While the specific toxic responses are the same regardless of exposure route (e.g. waterborne or dietary), the dose level, and time it takes to reach the threshold concentration body burden is likely to differ among species and potentially with exposure routes, and will depend on the rate of uptake, metabolism and elimination.

For example, in a study with rainbow trout fingerlings fed one of three dietary concentrations varying from 494 to 1,700,000 pg TCDD/g food for 71 or 91 days, toxic effects included reduced feeding activity after 10 days, growth effects after 30 days, and mortalities after 33 days, with mortalities reaching 50% after 61 days. However, the toxic effects were observed only in fish exposed to the highest concentration of 1,700,000 pg TCDD/g food. These concentrations resulted in a body burden in affected fish of 276,000 pg/g (wet weight). Residue concentrations in fish having no effect were 314 pg/g (Table 2.2, US EPA, 1993a and references therein).

In other studies, juvenile rainbow trout and yellow perch (*Perca flavescens*) fed diets containing 494 pg TCDD/g for 13 weeks accumulated tissue concentrations of 250 pg TCDD/g and 143 pg TCDD/g (whole fish), respectively (Table 2.2). However, these concentrations did not cause any observable effects in fin necrosis, cutaneous hemorrhage or increased mortality during the 26-week observation period. When dietary exposure was stopped, TCDD residues were slowly eliminated (Kleeman et al., 1986 a, b).

**Maternal transfer**

An important route of dioxin exposure to early life stage fish in the wild is expected to be through maternal transfer from the adult female to the developing eggs. Available data have shown maternal transfer of TCDD to eggs of between 39-50% of the maternal body burden in the whole fish.

A number of studies have been performed where adult fish are exposed to TCDD in contaminated food and the effects are observed on the early life stages following exposure via maternal transfer. In one study, adult brook trout were fed pellets containing TCDD for a period of 14 weeks, to achieve and maintain target tissue concentrations of 0, 75, 150, 300, 600 and 1,200 pg TCDD per g fish, up to the time of spawning (Table 2.3). Fish were fed uncontaminated food from the start of the spawning phase. Mean whole body concentrations in fish reached target levels at the end of the loading phase and remained

relatively constant throughout the maintenance phase. No treatment effects were observed in adult fish in terms of survival, growth, gross pathology, number of eggs produced, and fertility of eggs. The observed treatment effects involved biochemical changes including dose dependent increases in post spawn heptasomatic index (HIS) and decreases in post spawn leukocrits in both sexes. The biological significance of these changes is unclear. There was also delayed initial spawning in the highest treatment group (Tietge et al., 1998).

**Table 2.2 Summary of toxic effects on fish exposed to TCDD in food[9]**

| Species | Concentration in diet (pg/g) | Concentration in organism (pg/g) | Exposure/ Observation | Effect/endpoint |
|---|---|---|---|---|
| Rainbow trout fingerling | 3,290<br><br>1,700,00<br>494 | 314<br><br>276,000<br>250 | 71 d<br><br>71 d<br>91 d | No effect on survival or growth<br>100% mortality<br>No toxic effect |
| Lake trout adult | 22,000<br>22,000 | 59 (in eggs)<br>104 (in eggs) | 90 d/eggs to swim-up fry<br>90 d/eggs to swim-up fry | $LD_{50}$ (sac fry)<br>100% sac fry mortality |
| Zebra fish adult | 1,700<br>>8,300 | Not measured | Single dose/22 d<br>Single dose/1-2 spawnings | No effect<br>Reduced eggs per spawn, 100% larval mortality |
| Yellow perch juvenile | 494 | 143 | 13 wk/13 wk | No toxic effect |

**Table 2.3 Body burden in female brook trout and corresponding egg burden[10]**

| Maternal body burden pg/g whole body | Egg concentration pg/g egg wet wt | Effects in early life stages |
|---|---|---|
| 75 | 41 | No effect relative to controls |
| 150 | 84 | Elevated cytochrome P4501A1mRNA in 91 d postspawn, edema, exophthalmia in juvenile fish, hemorrhaging, edema |
| 300 | 156 | Edema, exophthalmia in juvenile fish, hemorrhaging |
| 326 | 127 | $LD_{50}$ early life stage fish |
| 600 | 285 | $LD_{100}$ |
| 1,200 | 517 | $LD_{100}$ |

[9] From US EPA 1993a, and references therein

[10] Following pre-spawn feeding with pellets containing TCDD (Johnson et al., 1998).

In the second phase of the study, mean TCDD concentrations in freshly spawned eggs following maternal transfer were determined to be 0 (controls), 41, 84, 156, 285 and 517 pg TCDD per g egg (wet weight), or approximately 39% of the adult female whole body TCDD concentrations (Table 2.3). After hatching, mortality began within 7 to 10 days in the two highest treatment groups and was nearly complete 20 days later. In the 156 pg TCDD treatment group, the onset of toxicity effects and mortality began later and progressed more slowly, with few additional deaths after 157 days. The concentrations in eggs causing 50% mortality ($LC_{egg50}$) at swim-up and at the end of the study were 138 and 127 pg TCDD per gram egg, respectively. Treatment induced pathologies occurring at lower doses included edema in free embryos from all treatment groups at hatch, increased prevalence of exophthalmia in juvenile fish ($EC_{egg50}$ = 117 pg/g), elevated levels of cytochrome P450 in 91-day postspawn free embryos, and hemorrhaging behind the eyes and at other locations. No treatment effects were observed on fertility, growth of alevins and post swim-up juveniles, or juvenile sex ratios (Johnson et al., 1998).

The measured TCDD concentration in eggs inducing cytochrome P450 in embryos was 58 pg TCDD per g egg, which was somewhat lower than the $LC_{egg10}$ of 88 pg TCDD per gram egg. This is different from the results reported for lake trout early life stages (Guiney et al., 1997), where detectable induction of CYP1A1 protein was caused by TCDD concentrations that were near or even slightly higher than the $LC_{egg50}$ (Johnson et al., 1998).

These studies indicate that, while adult fish exposed to TCDD in their diet may exhibit no toxic responses, exposure via maternal transfer may result in significant toxic effects in the more sensitive early life stages of the developing fish following hatching (Johnson et al., 1998; US EPA, 1993).

Johnson et al., 1998 predict that female brook trout with concentrations of 326 pg/g whole body would produce eggs containing 127 pg TCDD per g egg, which would cause 50% mortality of early life stage fish during development, while producing no toxic effects in adult fish. Thus, the sensitivity of early life stages to TCDD may effect successful recruitment in natural populations even when there are no obvious adverse effects observed in the adults (Tietge et al., 1998).

In another study with lake trout, adult females were fed pellets containing 22,000 pg TCDD/g for a month, three months prior to spawning. The diet was then changed to contaminated TCDD fathead minnow for the remaining period up to spawning. Eggs were fertilised by unexposed males. The sexually mature females exhibited a wide range of TCDD whole body burdens and associated egg burdens (<1 pg/g to 387 pg/g), which reflected differences in the amounts of food consumed between individual fish. Egg concentrations were approximately 50% of the value for whole fish. TCDD doses ≥233 pg/g resulted in inviable oocytes and failure of fertilisation, TCDD doses of 152 pg/g resulted in successful fertilisation. However, all sac fry that hatched died from blue-sac disease syndrome. The $LD_{50}$ (based on egg residue concentration) following maternal transfer was determined to be 59 pg/g (US EPA 1993a, and references therein).

In other similar studies, the reproduction and oogenesis of Zebra fish (*Brachio rerio*) were affected by a single dose of TCDD >8,300 pg/g. Results showed a rapid decrease in the

number of eggs produced per spawning, and after one to two spawning cycles, spawning ceased completely. Other effects included loss of adult body weight, and reduced activity 20-24 days after exposure. Hatched larvae developed cranial and thoracel edema, notochord malformations and larvae death after two to three days (US EPA 1993a, and references therein).

**Exposure by injection**

The effects of TCDD on several different fish species, exposed at the egg, fingerling or juvenile stage by administering the test substance in an injection into whole fish or fish eggs have been examined in several studies (Table 2.4).

Studies by Walker et al., (1991; 1992) found toxicity of TCDD to be only slightly higher for exposure by injection than for waterborne exposure. The $LD_{50}$ values for hatching and sac fry mortality in rainbow trout following 48 hours static waterborne exposure was 439 pg TCDD/g compared to 421 pg TCDD/g for egg injection. The $LD_{50}$ for lake trout following waterborne exposure was 65 pg/g, compared to 47 pg/g for egg injection (Walker et al., 1991).

In a series of studies, Walker and Peterson (1991) injected fertilised rainbow trout eggs with graded doses of TCDD, PCDD, PCDF and PCB congeners and then held fish eggs in clean water until swim-up. The calculated $LD_{50}$ values for TCDD, the most potent congener, ranged from 230-488 pg/g, based on the egg dose that caused mortality from hatching onset to swim-up. The highest mortality rate occurred in sac fry, and mortality was preceded by hemorrhages and fluid accumulation beneath the yolk sac epithelial membrane.

Van der Weiden et al., (1990) performed TCDD dose-response studies with rainbow trout to correlate enzyme induction (cytochrome P450) with toxicological effects. Trout received single TCDD injections of 10, 50, 100, 500, 1,000 or 5,000 pg/g. At a dose of 5,000 pg/g, 20% of fish died 11 and 12 weeks after exposure, and fish were characterised by poor growth, fin necrosis and abnormal behavior (head up swimming with hyperactivity followed by periods of immobility), increased liver weight and histopathological lesions. Increases in EROD activity and total cytochrome P450 content in the liver were TCDD dose-related and persisted above control levels for six weeks at 500 pg/g and 12 weeks for the 1,000 and 5,000 pg/g dose levels. EROD activity pattern paralleled effects found on growth and survival, except that both EROD induction in the liver and histopathological changes in the spleen were observed at the lower dose of 500 pg/g, a dose that did not cause toxic effects to these animals. In other studies van der Weiden et al., (1992, 1994) determined the LOAELs for EROD activity for rainbow trout and carp (*Cyprinus carpio*) to be 300 and 30 pg TCDD/g body weight, and the $ED_{50}$ values to be 910 pg TCDD/g bw in rainbow trout and 48 pg TCDD/g, respectively, bw in carp.

**Variations in fish species sensitivity**

While the toxic responses in fish following exposure to TCDD are similar across fish species and are consistent with the hypothesis that the mode of toxic action is the same across species, there is considerable variability in sensitivity among fish species. Results

provided in Table 2.5 show the NOAEL for sac fry mortality range between 34 pg/g and 1,190 pg/g, in fish eggs, which is a 35-fold difference in the effect concentrations.

**Table 2.4 Summary of toxic effects on fish exposed to TCDD by single injection[11]**

| Species | Concentration in organism (ng/g) | Duration Exposure/Observation | Effect/endpoint |
|---|---|---|---|
| Rainbow trout | | | |
| Eggs | 230 | fertilised egg to swim up | $LD_{50}$ (sac fry McConaughy strain) |
| | 240 | | $LD_{50}$ (sac fry Erwin strain) |
| | 374 | | $LD_{50}$ (sac fry Arlee strain) |
| | 488 | | $LD_{50}$ (sac fry Eagle Lake strain) |
| Fingerling | 1000 | 25 d | Significant hematological changes |
| | 5000 | 20 d | 20% mortality |
| | 5000 | 84 d | 20% mortality, increased liver weight |
| | 10000 | 80 d | $LD_{50}$ |
| Juvenile | 300-3,060 | 42-84 d | Fin hemorrhage, spleen histopathology, EROD & P4501A1 induction |
| | | 21 d | |
| | 790 | 72 h | $LD_{50}$ for EROD induction |
| | 640 | | $LD_{50}$ for AHH induction |
| Juvenile Carp (*Cyprinus carpio*) | 3000 | 80 d | $LD_{50}$ |
| Juvenile Bullhead (*Ictalurus punctatus*) | 5000 | 80 d | $LD_{50}$ |
| Juvenile Yellow perch (*Perca flavescens*) | 3000 | 80 d | $LD_{50}$ |
| Juvenile Largemouth bass (*Micropterus salmoides*) | 1,100 | 80 d | $LD_{50}$ |
| Juvenile bluegill (*Lepomis macrochirus*) | 1,600 | 80 d | $LD_{50}$ |

[11] From US EPA 1993a, and references therein

**Table 2.5 TCDD developmental toxicity in freshwater fish species**

| Species | Effect | Egg dose (pg/g ww) | Effect level | Reference |
|---|---|---|---|---|
| Lake trout | Sac fry mortality | 34<br>55<br>65 | NOAEL<br>LOAEC<br>$LC_{50}$ | Walker et al., (1991) |
| Brook trout | Sac fry mortality | 135<br>185<br>439 | NOAEL<br>LOAEC<br>$LC_{50}$ | Walker and Peterson (1994) |
| Japanese medaka | Lesions, etc.[1] | <100<br>300 | NOAEL<br>LOAEL | Wisk and Cooper, (1990) |
| Rainbow trout[2] | Sac fry mortality | 194<br>291<br>439 | NOAEL<br>LOAEL<br>$LC_{50}$ | Walker et al., (1992) |
| Lake herring | Sac fry growth and mortality | 175<br>270<br>902 (783-1,040) | NOAEL<br>LOAEL<br>$LC_{50}$ | Elonen et al., (1998) |
| Fathead minnow | Sac fry growth and mortality | 235<br>425<br>539 (476-661) | NOAEL<br>LOAEL<br>$LC_{50}$ | Elonen et al., (1998) |
| Channel catfish | Sac fry growth and mortality | 385<br>885<br>644 (576-721) | NOAEL<br>LOAEL<br>$LC_{50}$ | Elonen et al., (1998) |
| Medaka | Sac fry mortality | 455<br>949<br>1,110 (932-1,320) | NOAEL<br>LOAEL<br>$LC_{50}$ | Elonen et al., (1998) |
| White sucker | Sac fry growth and mortality | 848<br>1220<br>1,890 (1,760-2,030) | NOAEL<br>LOAEL<br>$LC_{50}$ | Elonen et al., (1998) |
| Northern Pike | Sac fry growth and mortality | 1,190<br>1,800<br>2,460 (2,100-2,880) | NOAEL<br>LOAEL<br>$LC_{50}$ | Elonen et al., (1998) |
| Zebra fish | Sac fry growth and mortality | 425<br>2,000<br>2,610 (2,310-2,950) | NOAEL<br>LOAEL<br>$LC_{50}$ | Elonen et al., (1998) |

[1]Consist of a spectrum of effects including hemorrhage in various areas, pericardial edema, collapse of the yolk sphere, cessation of blood flow throughout the animal, and embryo mortality;
[2]single egg injection.

The most sensitive species to TCDD exposure tested so far is the lake trout (*Salvelinus namaycush*). Brook trout are intermediate in their sensitivity to TCDD compared to lake trout and rainbow trout, following egg exposure. Rainbow trout sensitivity varies with the strain (Walker and Peterson, 1994). It is suggested that differences in the tolerance to TCDD between fish species may in part be due to differences in development rates and time to swim-up. Because TCDD is eliminated slowly from embryos and sac fry, fish with

longer development times retain TCDD for longer and are, therefore, exposed for longer (Elonen et al., 1998).

### 2.2.2 Epidemiological studies with wild fish

A number of field investigations have indicated a link between exposure to chemicals and reproductive failure and other toxic effects in fish populations. However, because a variety of chemical and non-chemical stressors can impact fish populations in the natural environment, it is often difficult to unequivocally determine the cause and effect relationships. Nevertheless, strong evidence for a link between TCDD contamination and declines in fish population comes from Lake Ontario, Lake Michigan, and Lake Huron in the Great Lakes of North America (see US EPA, 1993a, and US EPA 2002, Cook et al., 2003).

The loading of large amounts of dioxins into Lake Ontario during the middle of the 20$^{th}$ century coincided with a population decline and extinction of a number of fish species in the lake, beginning around the 1950 and 1960s. Species extirpated from the lake included lake trout (*Salvelinus namaycush*), burbot (*Lota lota*), and the deep-water sculpin (*Myoxocephalus quadricornis*). While these extinctions can be explained by many other factors such commercial over-fishing, predation and competition, eutrophication, and low dissolved oxygen; chemical contamination of sediments has been suggested as a likely contributor to the decline of fish populations, particularly the bottom dwelling, deep-water sculpin and Lake trout, a top trophic level predator fish (Cook et al., 2003). Chemical contamination has also been implicated in the poor reestablishment success of lake trout in Lake Michigan, Lake Huron, and Lake Ontario during the 1970s and 1980s. The field sampling record indicates no natural reproduction in Lake Ontario until 1986, most likely due to TCDD-related blue-sac disease. In salmonid sac fry, this blue-sac disease is caused by different physical and chemical stressors, including exposure to TCDD (US EPA, 1993a).

Lake trout eggs collected from Lake Ontario in 1981-1984 showed a very high incidence of blue-sac disease and associated mortality. The egg TCDD residue range (i.e. NOAEL) for this effect in laboratory studies with lake trout is 30 to 40 pg/g, wet weight, and the $LD_{50}$ for mortality of sac fry prior to swim-up is 40 to 70 pg/g, wet weight, body burden. The TCDD concentrations measured in lake trout eggs from Lake Ontario in the late 1980s indicated 7 to 16 pg/g, wet weight. Other PCDD, PCDF and PCB congeners also contributed to contamination. A TCDD toxicity equivalency concentration (TEC) in eggs of from 14 to 32 pg/g, wet weight, was estimated for other congeners. Older lake trout in Lake Ontario had up to three-fold greater whole body TCDD residues and estimated egg TECs of 42 to 96 pg/g, wet weight, which is significantly higher than the threshold and the $LD_{50}$ for mortality of sac fry (US EPA, 1993a).

Concentrations of organic pollutants in fish tissues have decreased by a factor of approximately 25 since maximum concentrations were reached in the lower Great Lakes in the mid-1970s. By 1988, TECs had decreased to 8 and 29 ppt (pg/g) in Lakes Michigan and Ontario, respectively. These concentrations are less than the threshold of approximately 40 ppt, but only slightly in the case of Lake Ontario (US EPA, 2002, and

references therein). In a recent study, Cook et al., (2003) suggested a threshold of 3 to 10 pg TEQ/g egg, based on $WHO_{98}$ fish TEFs, to prevent sublethal effects and to ensure good reproduction and recruitment of fish populations in Lake Ontario.

In an Australian field study, European carp (*Cyprinus carpio*) exposed to highly treated pulp mill effluent in Lake Coleman, Victoria, had significantly elevated (2.3 to 6.3 times) hepatic microsomal EROD levels relative to fish in nearby unexposed water bodies. EROD activity was correlated to low levels of PCDD/PCDFs measured in carp muscle (1.0 to 4.0 ppt TCDD, I-TEFs) compared to unexposed fish (0.48 to 0.64 ppt). Tissue concentrations of PCDD/PCDFs decreased with increasing distance from effluent point sources (Ahokas et al., 1994).

### 2.2.3 Saltwater fish

The toxic effects of TCDD in saltwater fish are similar to those observed in freshwater species and include delayed mortality following acute exposure, although there are fewer data available examining saltwater fish. The limited data suggest saltwater species are generally less sensitive to the toxic effects of TCDD than most freshwater species.

In one study described in US EPA (1993a), killifish (*Fundulus heteroclitus*) eggs were collected from TCDD contaminated and uncontaminated sites in New Jersey and New York, respectively, and were exposed to varying concentrations of TCDD from fertilisation to hatch. At concentrations of 200 ng/L, 20% of embryos from uncontaminated and 12% of embryos from contaminated died, while 55% of embryos from uncontaminated sites, and only 5.5% of embryos from contaminated had major lesions. The study indicated that previously exposed fish are less sensitive to TCDD exposure than unadapted fish. In a later study by Nacci et al., (2002), inherited tolerance to local contamination was also found in populations of estuarine killifish in Bedford Harbor, MA, USA.

In a similar study, Prince and Cooper (1995) examined the effects of TCDD exposure on estuarine killifish taken from TCDD contaminated and uncontaminated sites. Results showed fish from unpolluted sites exhibited characteristic responses to TCDD exposure including edema, hemorrhages, and arrested development culminating in death. However, deaths were not dose-dependent, with 75% of fish dead in the 30 ng/g treatment group and 55% dead in the higher 60 ng/g treatment group. Fewer deaths occurred in fish from contaminated sites, and these fish did not exhibit any TCDD treatment effects as observed in fish from unpolluted sites. This study concluded that killifish, *Fundulus heteroclitus,* is the least TCDD-sensitive fish so far studied for both embryonic and adult life stages.

In other studies, winter flounder (*Pleuronectes americanus*) administered 4,500 pg/g TCDD twice by stomach intubation, showed a significant increase in EROD activity after eight days of treatment. Increases in the activity of this enzyme are indicative of xenobiotic activity with the Ah receptor, induced particularly by TCDD (US EPA 1993a, and references therein).

Another flounder species, *Platichthys flesus*, administered TCDD twice within seven days at five concentrations between 10 and 100,000 pg TCDD/g body weight showed TCDD dose-related effects in hepatic cytochrome P450 induction and associated increased EROD

activity. The LOAEL for EROD activity in flounder was determined to be 680 pg TCDD/g body weight (360 pg TCDD/g liver), while the $ED_{50}$ was 1610 pg TCDD/g bw, (1290 pg TCDD/g liver). No changes were observed in liver retinoid, plasma retinol, and plasma thyroid hormone parameters, and no induction was observed of biotransformation enzymes associated with chemical exposure, such as glutathione-*S*-transferase activity. There was in addition, no TCDD-induced change in body and liver weight. The study concluded that, while flounder are an Ah receptor-responsive species with respect to CYP1A induction, they did not exhibit Ah receptor toxic responses from exposure to TCDD similar to other fish and mammalian species (Besselink et al., 1997). The $LD_{50}$ and LOAEL for EROD induction indicate flounder are much less sensitive than rainbow trout and carp to TCDD exposure.

### 2.2.4 Freshwater algae and plants

Limited studies in aquatic model ecosystems indicate that plants are less sensitive to TCDD than most species of animals following similar exposure periods (US EPA 1993 and references therein). Measured water concentrations of up to 1.33 μg/L had no observable effects on algae (*Oedogonium cardiacum*) and duckweed (*Lemna minor*) during a 33-day exposure period, while measured residues in algae were 2,300,000 pg/g after 33 days. Duckweed exposed to TCDD concentrations up to 7.13 ng/L had residue levels of 307,000 pg/g, with no toxic effects.

### 2.2.5 Freshwater and marine invertebrates

The US EPA (1993a) lists only six endpoints for freshwater invertebrates determined in studies conducted in the 1970's. In these studies, no mortalities or reproductive effects were observed when snails (*Physa sp*.), annelid worms (*Paranais sp*.), and mosquito larvae (*Aedes aegypti*) were exposed to 200 ng/L (nominal) TCDD in water under static conditions for 36, 55 and 17 days, respectively. Similarly, no mortalities, growth or reproductive effects were observed when water flea (*Daphnia magna)* and snails (*Physa sp*.) were exposed in a model ecosystem for 33 days to 1,300 ng/L TCDD, while attaining residue concentrations of 1,570,000 pg/g, and 502,000 pg/g, respectively. No toxic effects were observed up to seven days after *Daphnia magna* were exposed for 48 hours under renewal conditions to 1,030 ng/L TCDD.

In a more recent study, two species of freshwater benthic invertebrates, *Chironomus tentans* and *Lumbriculus variegates,* were exposed to dietary concentrations of 30, 300 and 3,000 ng (nominal) TCDD over their full life cycle. No toxicity or adverse effects on growth and reproduction were observed, despite residues in tissues reaching concentrations of 6,876,000 pg/g and 9,533,000 pg TCDD/g lipid in *C. tentans* and *L. variegatus,* respectively. Depuration studies indicated elimination followed first-order kinetics. Half-lives ranged from 314 to 495 h for *L. variegatus* and from 70 to 99 hours for *C. tentans* (West et al., 1997).

Barber et al., (1998) found no significant effects of acute TCDD exposure on the survival or growth, relative to controls of the intertidal marine amphipod, *Ampelisca abdita*. In this study, sediments were spiked with TCDD at concentrations of up to 25,000 pg/g dry

weight, equilibrated for 14 days, prior to conducting the 10-day bioassay. The TCDD concentrations in amphipod tissue were not determined in this study. In another study, Rubenstein et al., (1990) reported no effects in sandworms, grass shrimps, or clams after exposure to sediment containing an average of 660 pg/g TCDD.

### 2.2.6 Amphibians and reptiles

**Amphibians**

Only a limited number of studies have examined the effects of TCDD exposure on amphibians. In one study, tadpoles and bullfrogs (*Rana catesbeiana*) were injected with TCDD at doses of 25,000 to 1,000,000 pg/g, and 50,000 to 500,000 pg/g body weight, respectively. No mortalities among tadpole or adult frogs were observed for up to 50 and 35 days after exposure, respectively. All surviving tadpoles successfully completed metamorphosis with no morphological abnormalities. Histopathological examination of liver, heart, kidney, lung and reproductive organs indicate no lesions (US EPA 1993a, and references therein).

Jung and Walker (1997) exposed three species of frog, at the egg and tadpoles stages, to waterborne and vehicle controlled (0.7% acetone) TCDD for 24 hours, and then subsequently raised them in clean water. The America toad (*Bufo americanus*) was exposed to concentrations of 3 to 30,000 ng/L TCDD, the leopard frog (*Rana pipiens*) was exposed to 3000 ng/L TCDD, and the green frog (*Rana clamitans*) was exposed to 300-100,000 ng/L TCDD. There were relatively few impacts of acute exposure to TCDD on either eggs or tadpoles. The only significant TCDD related mortality was a 10% increase in leopard frog egg and early tadpole mortality exposed to 3000 ng/L TCDD. No significant morphological abnormalities were observed, except for an increased occurrence of lighter pigmentation in leopard frog and green frog tadpoles. American toad, leopard frog, and green frog eggs accumulated ranges of one to four, four to seven, and one to three times the nominal TCDD water concentrations, respectively. However, eggs and tadpoles eliminated TCDD relatively quickly, with half-life times from one to seven days.

Amphibians appear to be about 100- to 1,000-fold less sensitive to the deleterious effects of TCDD than the early life stages of fish. Reduced sensitivity of anuran amphibians (frogs) to TCDD compared to fish can be explained by the more rapid embryonic development in frogs (Jung and Walker, 1997). Amphibian eggs hatch within three to six days of laying, compared to 60 days for lake trout. Further, salmonid alevins retain and feed from the yolk sac containing maternal lipids for 120 days after hatching and so the dose received from the mother's body burden is much greater.

**Reptiles**

No laboratory toxicity data are available for reptiles. However, a small number of studies are available correlating dioxin levels and biological parameters in the snapping turtle (*Chelydra serpentina serpentine)*. Bishop et al., (1991), found a strong association between PCBs levels in snapping turtle eggs from contaminated sites in the Great Lakes and deformities and hatching success in the turtles, although the presence of other organochlorine chemicals in the eggs was a confounding factor. Snapping turtle eggs were

collected between 1986 and 1989 immediately after oviposition from four locations in the Great Lakes and from an uncontaminated reference site (Algonquin Provincial Park in north-central Ontario). Eggs were analysed for hatching success, incidence of deformities, and contaminant concentrations. A total of 202 clutches were artificially incubated for the study of embryos, hatchlings, and hatching success. The highest incidence of deformities, including deformities of the tail, hind legs, head, eyes, scutes, forelegs, dwarfism, yolk sac enlargement, and missing claws occurred in turtles from the most contaminated, Lynde Creek (total PCBs = 2,708,000 pg/g) and Cootes Paradise (total PCBs 25,000-3,322,000 pg/g). The most common deformity, occurring at all sites was abnormality of the tail. Hatching success was lowest in Cootes Paradise with the mean number of unhatched eggs/egg ranging from less than 0.1 to greater than 0.4 between 1986-88. The least contaminated site, Algonquin Park, showed the greatest hatching success with the mean number of unhatched eggs/egg less than 0.1 each year (Bishop et al., 1991).

In a later study, Bishop et al., (1998) collected snapping turtle eggs from seven study locations between 1989 to 1991 in the Great Lakes basin and St. Lawrence River, and Algonquin Provincial Park, and the eggs were artificially incubated. The incidence of abnormal development (including curled, bent, twisted, or absent tail; shortened or absent legs or digits; deformed eyes; recessed lower jaw, reduced body size; undeveloped carapace; presence of absence of scutes; and unresorbed yolk sac) increased significantly with increasing concentrations of polychlorinated aromatic hydrocarbons, particularly PCDD and PCDF, yet these were not correlated with TEQs in eggs. Mean total PCB concentrations in eggs were 241,000-3,950,000 pg/g, wet weight at the study sites and 18,000 pg/g at the reference site. Individual PCDD congener concentrations ranged from 2.6 to 36.4 pg/g egg, and PCDF concentrations ranged from 0.2 to 30.6 pg/g egg. The percentage of unhatched eggs, due to infertility or early interruption of embryonic development, ranged from 0 to 10% for all but a single clutch in which 40% of eggs failed to hatch. EROD and cytochrome P4501A activities were significantly higher in the liver of hatchlings from Lake Ontario compared to the reference site. Porphyria was not observed in turtles from either site (Bishop et al., 1998).

TCDD has been reported to have deleterious effects on endocrine and reproductive systems in alligators. In a study by Matter et al., (1998), approximately 250 American alligator (*Alligator mississippiensis*) eggs were collected from Bear Island Wildlife Management, Beaufort County, South Carolina. Eggs were injected with graded doses of TCDD between 100,000 and 10,000,000 pg/g egg, incubated at male-producing temperatures (32.5-34ºC), and reared to 21 days. Eggs treated with TCDD exhibited dose-dependent alterations in sex ratio, with a significant occurrence of female gonadal differentiation and numbers of females produced at male producing temperatures. TCDD exposure reduced both the size and number of vacuoles, and testes exhibited masses of abherent cells in the lumen of seminiferous tubules.

## 2.3 Summary of aquatic toxicity

Dioxins are highly toxic to both freshwater and marine fish, particularly early life stage fish, although the saltwater fish species tested so far are more tolerant to TCDD exposure than most freshwater species. Previously exposed saltwater fish are also more tolerant than unadapted fish.

Typical toxic responses in early life stage fish following exposure to TCDD include yolk sac edema, hemorrhaging, arrested development, and head and spinal deformities, and responses in older fish include lesions, fin necrosis and death. The toxic effects of dioxins occur as a function of body burden, with the effects often manifesting some time after the initial exposure period has ended when tissue residues have reached a particular threshold concentration for a given toxic response.

There is considerable variability in sensitivity to dioxins among fish species, with the NOAEL for sac fry mortality following embryo exposure ranging between 34 pg/g and 1,190 pg/g, in fish eggs, which is a 35-fold difference in the effect concentrations. The salmonid fish are the most sensitive class of fish, and the most sensitive species is the lake trout. The $LD_{50}$ for sac fry mortality in lake trout is 65 pg TCDD/g and the NOAEL is 34 pg TCDD/g egg.

The difference in the tolerance to TCDD between fish species is in part due to differences in development rates and time to swim-up. Fish with longer development times, retain TCDD for longer and are, therefore, exposed for longer. Elimination of TCDD by Lake trout embryos is slow, and so embryos and sac fry retain most of the maternal dose during sensitive early life stage development.

Amphibians appear to be about 100- to 1,000-fold less sensitive to the deleterious effects of TCDD than the early life stages of fishes. Reduced sensitivity among amphibians compared to fish can be explained by the more rapid embryonic development in frogs.

Studies indicate that aquatic plants and invertebrates including midges, cladocerans, sandworms, snails, grass shrimps, and amphipods are not sensitive to the toxic effects of dioxins. The observed lack of sensitivity of aquatic plants, and freshwater and marine invertebrates is consistent with the view that the Ah receptor is not present in invertebrates. However, many amphibians, plants and invertebrates have the ability to accumulate relatively high concentrations of some dioxin-like congeners in their bodies, while experiencing no toxic effects. Therefore, amphibians, aquatic invertebrates and plants are potential sources of exposure to higher organisms consuming them for food.

The sensitivity of reptiles is not well known. Limited data show that snapping turtles and alligators exposed to dioxins exhibit reproductive and developmental toxicity.

## 2.4 Avian Toxicity

Toxicological data available for birds include laboratory studies performed with a limited number of species exposed to dioxins via injection or in their diet, and a comparatively larger database of studies conducted with bird eggs exposed to dioxin via injection. In addition to the laboratory studies, epidemiological data are available examining dioxins in birds from wild populations including top predators in the aquatic food chain. These studies have usually involved collecting birds or bird eggs from wild populations located in known contaminated sites to correlate environmental and tissue concentrations to biological effects as observed in the natural environment.

Data are also available from *in vitro* studies. These studies are typically performed using extracts from the eggs of wild bird populations living in contaminated areas or using extracts from dosed laboratory animals to assess toxicity, and often to determine the relative potencies (commonly to liver cells) of different dioxin congeners for the purposes of deriving TEFs.

### 2.4.1 Laboratory studies

**Dietary exposure**

Birds are likely to be exposed to dioxins in the natural environment in their food, or by consuming contaminated soil. Limited studies are available where adult or juvenile birds have been exposed to dioxins in their diet. Dietary exposure to dioxins has been shown to cause death and chick edema disease in domestic chickens. Clinical signs of this disease include dyspnea, reduced body weight gain, stunted growth, subcutaneous edema, pallor and sudden death (WHO, 1989).

Galliformes (domestic chickens, pheasants, quails, grouse, turkeys etc.) appear to be the most sensitive order of birds, with the domestic chicken the most sensitive species so far tested. In a dietary study, the relative potency of three different PCDD congeners (TCDD, hexaCDD, octaCDD) for inducing chick edema disease was examined in laboratory studies with 3-day old white leghorn cockerels (*Gallus gallus*). Edema was induced in chicks fed 1000 or 10,000 pg TCDD/g body weight per day for 21 days, and in chicks fed 10,000 or 100,000 pg hexaCDD/g body weight per day. Chick edema was not observed in chicks fed a diet of 0.1 or 0.5% octaCDD for 21 days (WHO, 1989, and references therein). In another dietary exposure study, domestic chickens given a single dose of TCDD at 25,000-50,000 pg/g bw died within 12 to 21 days after treatment (US EPA, 1993a).

A few other bird species have been exposed to TCDD in their diet, and these species are less sensitive than chickens to the toxic effects, although the bobwhite quail, another galliforme, has a similar magnitude of sensitivity as the domestic chicken. In studies described in US EPA (1993a), the 37-day $LD_{50}$ values for bobwhite quail (*Colinus virginianus*), mallard duck (*Anus platyryhchus*), and ringed turtle dove (*Stretopelia risoria*), orally administered a single dose of TCDD were 15,000 pg, >108,000 pg and >810,000 pg TCDD/g body weight, respectively. In all of these studies, a dose-dependent decrease in

food consumption and body weight preceded death (US EPA, 1993a, and references therein).

In another study, eggs from the common tern (*Sterna hirunda*) were collected from breeding colonies in the Netherlands and were artificially incubated until hatched. The hatchlings were subsequently fed fish contaminated with either PCB 126 or PCB 126 and PCB 153 for 21 days (Bosveld et al., 2000). The resulting concentrations in food were from 10 pg to 1,200 pg TEQ/g wet weight. TEQs were calculated using two TEF systems (S-TEFs, and A-TEQs) after the methods of Safe (1990, 1994), Kennedy et al., (1996b), and Bosveld et al., (1992).

Results showed the LOAEL for biochemical and other effects (i.e. increased EROD activity, 50% reduction in plasma total thyroxine, decreased bursa weight) was 25,000 pg TEQ/g, liver lipid, caused by concentrations in food of about 600 pg TEQ/g fish wet weight. This concentration in food is six times higher than the NOAEL for mortality of 100 pg TCDD/g/d determined for three day old white leghorn chickens exposed for 21 days chickens to doses between 10-10,000 pg/g/d.

**Embryo exposure via injection**

A relatively large number of studies have exposed birds to dioxins via injection. Most of these studies have been performed with eggs, with only a small number being performed with adult or juvenile birds. These studies show that bird embryos are more sensitive than adults and hatchlings to the toxic effects of dioxins. There is also considerable variability among species in their sensitivity and response to dioxins in egg exposure studies.

In one of the few injection studies with adult birds, ring-necked pheasants (another Galliforme) were given a single injection of TCDD at doses between 6250 and 100,000 pg/g. All birds treated with the highest dose died within six weeks. The $LD_{80}$ was determined to be 25,000 pg/g, and the NOAEC for mortality was 6,250 pg/g (Nosek et al., 1992a).

The LOAELs causing embryo mortality and developmental effects range between 10 and 10,000 pg/g fresh weight TCDD in bird eggs for the most and least sensitive species, respectively (Table 2.6). The domestic chicken is again the most sensitive species, while the eastern bluebird (a Passerine – perching bird) is the least sensitive species tested.

Powell et al., (1996) injected the yolks of White Leghorn chicken eggs prior to incubation with graded doses of PCB 126 ranging from 100 to 12,800 pg/g egg, and with doses of TCDD ranging from 40 to 640 pg/g egg. Chicks were sacrificed within 24 hours of hatching for analysis of toxic responses. Both TCDD and PCB 126 caused embryo mortality. The $LD_{50}$ values for embryo mortality were 2,300 ± 190 pg/g egg for PCB 126 and 150 ± 12 pg/g egg for TCDD. Other significant effects included increased developmental abnormalities of the skull, beak, eyes and toes, and changes in body and organ weights, and edema.

Injection of TCDD into fertilised eggs of the ring-necked pheasant (*Phasianus colchicus*) produces embryo mortality, but no other signs that are classic to exposure of TCDD seen in chicken embryos. In one study, fertile pheasant eggs were injected (into albumin and yolk)

with graded doses between 0.1-100,000 pg TCDD/g egg. Embryonic developmental toxicity was assessed in one-day old hatchlings and in 28-day old chicks.

Egg TCDD doses as high as 1.0 µg/kg had no effects in one-day old hatchlings or 28-day old chicks in terms of body growth, organ weights, carcass morphometrics, edema, cardiac malformations and histological alterations (Nosek et al., 1993). The $LD_{50}$s for chick mortality were 1,354 pg/g for albumin and 2,182 pg/g for yolk injections (Table 2.6). Developmental abnormalities including beak deformities are a common response observed in different bird species following exposure to dioxins.

**Table 2.6 Developmental toxicity in bird embryos following a single egg injection**

| Species | Effect | Egg dose | Effect level | Reference |
|---|---|---|---|---|
| Chicken | Embryo mortality | 2,300 pg/g PCB 126<br>64 pg TEQ/g[1] | $LD_{50}$ | Powell et al., (1997) |
| Chicken | Embryo mortality | 240 pg TCDD/g | $LD_{50}$ | Allred and Strange (1977)[4] |
| Chicken | Embryo mortality | 150 pg TCDD/g | $LD_{50}$ | Powel et al., (1996b) |
| Chicken | Cardiac malformations | 9 pg TCDD/g[1] | LOAEL | Cheung et al., (1981a,b)[4] |
| Ring-necked pheasant | Embryo mortality | 2,182 pg TCDD/g[2] | $LD_{50}$ | Nosek et al., (1993) |
| Ring-necked pheasant | Embryo mortality | 1,354 pg TCDD/g[3] | $LD_{50}$ | Nosek et al., (1993) |
| Ring-necked pheasant | Embryo mortality | 1,000 pg TCDD/g | LOAEL | Nosek et al., (1993) |
| Ring-necked pheasant | Embryo mortality | 100 pg TCDD/g | NOAEL | Nosek et al., (1993) |
| Eastern bluebird | Embryo mortality | 10,000 pg TCDD/g | LOAEL | Thiel et al., (1988)[4] |
| Eastern bluebird | Embryo mortality | 1,000 pg TCDD/g | NOAEL | Thiel et al., (1988)[4]; Martin et al., (1989)[4] |
| Double-crested cormorant | Embryo mortality | 177,000 pg/g PCB 126<br>4,000 pg TCDD/g | $LD_{50}$ | Powell et al., (1998) |

[1]TEQ based on contaminated egg extracts
[2]Injected into the egg yolk
[3]Injected into the egg albumin
[4]Reference not seen, cited from US EPA 2000d

Hoffman et al., (1998) compared the toxic effects of PCB 126 and PCB 77 in the White leghorn chicken (*Gallus gallus*), American kestrel (*Falco sparverius*), and common tern (*Sterna hirunda*) by exposing eggs through injection prior to hatching. PCB 126 caused malformations and edema in chickens starting at 0.3 ppb (ng/g), in kestrels at 2.3 to 23 ppb, and in terns at levels 44 ppb. The extent of edema was most severe in chickens and least in terns. Beak deformities occurred in all three species, but crossed beaks were more prevalent in terns, while adverse effects on embryo growth were most apparent in chickens and kestrels. The $LD_{50}$ values for PCB 126 were 0.4 ppb for chickens, 65 ppb for kestrels, and 104 ppb for terns. The $LD_{50}$ values for PCB 77 were 2.6 ppb in chickens, and 316 ppb in kestrels. If we assume the $WHO_{98}$ relative potency of 0.001 for PCB 126 compared to TCDD, these values would equate to $LD_{50}$ values of 0.4 pg TCDD/g for chickens, 65 pg TCDD/g for kestrels, and 104 pg TCDD/g for terns.

Powell et al., (1997) conducted egg injection studies with double-crested cormorants (*Phalacrocorax auritus*), which involved injecting eggs collected from wild populations from uncontaminated sites with PCB 126 (5,000-800,000 pg/g wet weight) and TCDD (60-4,000 pg/g wet weight), or with an extract derived from field-collected cormorant eggs from contaminated sites. A median $LD_{50}$ for PCB 126 of 158,000 pg/g egg was derived for cormorants based on embryo mortality. Significantly greater mortality occurred in embryos exposed to TCDD at the highest test dose of 4,000 pg/g egg, however, no $LD_{50}$ for TCDD could be derived owing to insufficient mortality information. In a follow up study, Powell et al., (1998) injected double-crested cormorant eggs prior to incubation with PCB 126, and with higher doses of TCDD (i.e. 1,300-11,700 pg/g egg) than in the previous study. Median lethal doses calculated from mortality data at hatching were 177,000 pg/g egg for PCB 126, and 4,000 pg/g egg for TCDD. Other adverse effects included increased bursa weight and elevated hepatic EROD activity. No significant differences were observed in body weight, or developmental abnormalities, gonad histology or sex ratio in any treatment groups. The above results indicate double-crested cormorants are significantly less sensitive to dioxin-like toxicity than White Leghorn chickens. The cormorant is approximately 30 times less sensitive to TCDD, and 80 times less sensitive to PCB 126 than chickens.

Sensitivity to biochemical changes following exposure to dioxins varies among different bird species. Cytochrome P4501A induction in response to various PCBs, TCDD and TCDF was measured in primary hepatocytes from several avian species to determine its usefulness as a tool to measure relative sensitivity (Kennedy et al., 1996b). Among species, the rank order in sensitivity to EROD induction is chicken > pheasant > turkey > mallard duck > herring gulls. Relative sensitivity rankings of species following administration were similar to those obtained by measuring lethality during *in ovo* (in egg) dosing studies. In *in ovo* studies, the rank order in sensitivity to TCDD is chicken > pheasant > turkey > mallard duck > domestic duck > domestic goose > herring gulls > black-headed gull > eastern bluebird (US EPA, 2000d).

**Maternal transfer**

Nosek et al., (1992b) exposed adult ring-necked pheasant to TCDD to predict embryonic toxicity following exposure via maternal transfer from adult females birds to eggs. At the time of egg formation, dioxins in adult birds are transferred along with lipid into the eggs. In eggs, dioxins are taken up in the blood and distributed throughout the egg, with the majority being stored in the lipid-rich egg yolk (Bosveld et al., 1992). It is estimated that about 1% of the adult body burden of TCDD in hen pheasants is translocated to each egg during production, with essentially all of the TCDD in the egg being contained in the yolk (Nosek et al., 1992b).

More than 98% of embryos died in eggs laid by pheasant hens exposed prior to pairing with roosters to a dose of 1,000 pg/g TCDD per week for 10 weeks (resulting in a cumulative dose of 10,000 pg/g). Hens also displayed reduced egg production, and 57% of hens died. No deaths were observed in hens exposed to lower doses of 10 and 100 pg TCDD/g body weight. TCDD was found to be highly persistent in hens, with a half-life for whole body elimination of about 378 days. Based on a pheasant weight of 1 kg and a body burden of 10,000 pg/g, it was estimated that the eggs contained 100,000 pg TCDD/egg. For an egg weighing 30.5 g, this equated to a dose of 3,300 pg TCDD/g egg (Nosek et al., 1992 a, b).

In another study, Alonso et al., (1998), injected White leghorn chickens with TCDD and followed offspring through their development to assess alterations in reproductive maturity. First generation (F1) adult females exposed to maternal doses of 8,600 pg/day began egg production approximately two weeks later than F1 control adult females, and produced fewer eggs; however, by week eight, egg production was the same between groups. No long-term effects on reproductive rates were evident.

### 2.4.2 Epidemiology studies with wild birds

The Great Lakes regions of Canada and the USA have been the focus of a number of epidemiological studies with wild bird populations residing on the lakes. These studies typically involved collecting eggs from wild populations in known contaminated sites, incubating the eggs, and measuring chemical concentrations in tissues, organs and eggs for correlation with biological effects. These data are provided in Table 2.7, along with data from additional studies.

The Great Lakes have suffered historical contamination predominantly from municipal and industrial wastes released into the lake, particularly since World War II (Colborn and Smolen, 1996). PCDDs, PCDFs and PCBs, along with other contaminants such as dichlorodiphenyldichloroethylene (DDE), have been detected in sediments, fish and wildlife associated with the lake. Colonial, fish-eating birds nesting along the shoreline of the lakes exhibit poor breeding success compared to populations nesting on uncontaminated water bodies. Birds in the region display symptoms similar to those associated with exposure to TCDD and other chlorinated chemicals, with the observed effects being strongly correlated with concentration TEQs of planar dioxin-like compounds. These adverse effects include embryo lethality and developmental abnormalities such as beak deformities and chick edema disease (Giesy et al., 1994).

**Table 2.7 Summary of studies with wild birds examining exposure to TCDD or other dioxin-like chemicals**

| Species | Effect | Exposure | Egg concentration | Effect level | Reference |
|---|---|---|---|---|---|
| Bald eagle | Hepatic CYP1A induction | Eggs collected from wild colonies near pulp mills, Southern British Columbia | 100 pg/g egg TEQ<br>210 pg/g egg TEQ (Ahlborg *et al* TEFs) | NOAEL<br>NOAEC | Elliot *et al* (1996)<br>Elliot *et al* (2001) |
| White-tailed sea eagle | Threshold liver concentration | Wild birds collected between 1979-1998 from Eastern Germany | 1,500 pg TCDD/g liver wwt | NOAEL | Kannan *et al* (2003) |
| White-tailed sea eagle | Reduced productivity | Wild birds, collected from Baltic Sea (egg and muscle concentrations) | <1,200 pg/g TEQ<br><1,040 pg/g TEQ<br>7 pg/g egg | Not reported; PCBs 75% TEQ<br>Threshold level | Koistinen *et al* (1997) |
| Osprey | Embryo mortality<br>Hepatic CYP1A induction | Eggs collected downstream of bleached-kraft mills in Wisconsin, USA | 136 pg/g TEQ egg<br>130 pg/g TEQ egg | NOAEL<br>LOAEL | Woodford et al., (1998)<br>Elliot *et al* (2001) |
| American kestrel | Malformations and edema | Bird eggs from captive colony in wildlife research centre exposed by injection | 2.3 to 23 (µg/kg) ppb PCB 126 | $LD_{50}$ = 65 µg/kg PCB 126<br>$LD_{50}$ = 65 pg/g TEQ | Hoffman *et al* (1998) |
| Albatross | Reproductive success | Wild birds eggs from Midway atoll, North Pacific | 52 ng TCDD Teq/kg ww egg (Laysan albatross), 124 TCDD Teq/kg (Black-footed albatross) | Based on PCB concentrations. TEFs based on Ahlborg and Hanberg (1994) | Auman *et al* (1997) |
| Wood duck | Reduction in egg hatching | Arkansas wetland with point source contamination | 36 pg/g egg,<br>14 pg/g<br>4.2 pg/g,<br>0.01 pg/g<br>20-50 pg/g TEQ<br>70 pg/g | 47% hatched<br>62% hatched<br>79% hatched<br>93% hatched<br>Threshold range<br>$LD_{50}$ | White and Seginak (1994) |

**Table 2.7 Continued**

| Species | Effect | Exposure | Egg concentration | Effect level | Reference |
|---|---|---|---|---|---|
| Double-crested cormorant | Mortality | Egg injection | 4,000 pg/g TCDD egg | $LD_{50}$ | Powell *et al* (1998) |
| Double-crested cormorant | Reduced productivity | Eggs collected from wild colonies in contaminated area (Netherlands) | 10-100 μg/g PCB<br>0.8-2 pg/g PCDD, PCDF | 5 fold increase in EROD activity; 50% increase plasma FT4;<br>15-20% reduction in head length | Van den Berg et al., (1994) |
| Double-crested cormorant | Poor breeding success, egg mortality | Eggs collected from 11 wild colonies in Great Lakes, USA | 35-344 pg/g TEQ egg | 8-39% egg mortality correlated to TCDD TEQ (H4IIE) | Tillitt *et al* (1992) |
| Double-crested and pelagic cormorant | Measured concentrations and related findings to published data | Eggs collected from wild colonies in contaminated area, Canada (1973-1998) | All eggs contained TEQ ≥ 148 pg/g egg | Elevated EROD and brain asymmetry | Harris *et al* (2003) |
| Great blue heron | Depression of growth compared to uncontaminated site | Eggs collected from wild colonies in contaminated area, British Columbia | 135 ± 49.6 pg/g<br>11 ± 33.7 pg/g | Not reported | Hart *et al* (1991) |
| Great blue heron | Increased edema, decreased body weight | Hatchlings from British Columbia, Canada | 100-278 pg TEQ/g | Not reported | Sanderson et al., (1994). |
| Great blue heron | Biochemical and morphological effects | Wild colonies in contaminated area, British Columbia | 472 pg TEQ/g ($WHO_{98}$ TEFs) | Levels not reported<br>No reduction in survival | Elliott et al., (2001) |

**Table 2.7 Continued**

| Species | Effect | Exposure | Egg concentration | Effect level | Reference |
|---|---|---|---|---|---|
| Common tern | Hatching success, beak malformations and edema | Collected from uncontaminated site, Barren Is, Chesapeake Bay, USA, exposed by injection | 44 ppb (µg/kg) PCB 126 | $LD_{50}$ = 104 µg/kg | Hoffman *et al* (1998) |
| Common tern | CYP1A induction, bursa weight | Hatchlings from eggs collected from breeding colonies (Netherlands) fed contaminated fish (PCB 126, PCB 153) | 25 µg TEQ/kg liver lipid (fish concentration 0.6 µg TEQ/kg fish ww) | LOAEL | Bosveld et al., (2000) |
| Caspian tern *(Hydroprogne caspia)* | Embryo mortality | Eggs collected from various locations in Great Lakes Region. | 750 pg/g H4IIE TEQ | $LD_{50}$ | Giesy *et al* (1994) |
| Foster's Tern | Increased incubation, reduced hatchability, increased liver weight, edema, congenital abnormalities | Eggs collected from wild colonies Great Lakes | 8 pg/g TCDD egg (Lake Poygan)<br>37.3 pg/g TCDD egg (Green Bay) | Not reported | USEPA (2000c)<br>Tillitt *et al* (1993) |
| Tree Swallow | Reproductive success, growth & development | Eggs and nestlings collected from wild colonies in the PCB contaminated upper Hudson River, USA | Concentration in eggs and nestlings 410-25,400 pg/g $TEQ_{bird}$ | Enlarged clutch sizes, increased number of infertile eggs, reduced hatchability, high nest abandonment rate | Secord *et al* (1999);<br>McCarty and Secord (1999) |

The contaminant concentrations in the Great Lakes reached a maximum in the 1970s, and have since declined significantly owing to bans and restrictions placed on the use and release of many of the compounds. Some bird populations, such as double crested cormorants and herring gulls have made dramatic recoveries since then, while other species, such as common and Forster's tern and bald eagle continue to decline (Giesy et al., 1994). The current rate of decline in contaminant levels has slowed down, caused in part by inputs from reservoir sources (e.g. stored in sediment and organisms) and from long-range transport (US EPA, 2002). The tissue of fish-eating water birds still contain PCBs, PCDDs and PCDFs at concentrations high enough to account for the adverse effects in the birds and their chicks despite the decreases in contaminant levels.

**Double-crested cormorant**

Double-crested cormorant populations on the Great Lakes experienced reproductive impairment from the late 1950s through to the 1970s, and although populations have increased in recent years, they continue to exhibit embryo lethality. The adverse effects are correlated to consumption of contaminated fish from the Great Lakes (Powell *et al* 1997). Developmental abnormalities in double-crested cormorants have been observed at Green Bay (WI, USA). The average concentrations of contaminants measured in the eggs of the cormorants from Green Bay were 1,300 pg TEQ/g, 20 pg TCDD/g, and 3,600 pg PCB 126/g. The rate of occurrence of beak deformities in the cormorant colony ranged from 0.5 to 0.9% (Powell et al., 1998, and references therein).

Tillitt et al., (1992) found a strong correlation between egg mortality and TCDD-EQ[12] (based on the H4IIE rat hepatoma assay) in double crested cormorant eggs collected from 11 colonies at five regional areas of the Great Lakes of North America. Egg mortalities between 8 and 39%, were associated with TEQs ranges between 35 and 344 pg/g. Planar PCBs accounted for most of the dioxin equivalents.

Some researchers have argued that the nesting success and incidence of deformities in cormorants is related to DDE concentrations rather than PCB concentrations and TEQs. For example, Custer et al., (1999) found a correlation between the levels of the DDT metabolite, DDE, and the hatching success of cormorants at Green Bay, but found no correlation between PCB concentrations and hatching success or EROD activity. These controversies illustrate the difficulty of assigning causes to adverse effects observed in natural environments, where multiple chemical and other potential stressors are present.

The Great Lakes is not the only region where organic chemical pollutants are associated with poor breeding success in water birds. Cormorant populations in the Netherlands

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[12] TCDD-EQs are TCDD toxic equivalents determined from the sensitive H4IIE *in vitro* bioassay test. In this bioassay extracts from tissue (e.g. eggs) that contain complex mixtures of dioxins are used to induce specific cytochrome P450–requiring mixed oxygenase enzymes induction in cultured rat hepatoma cells (H4IIE). Induction of enzymes in these cells is an integrative measure of the toxic potency of the mixtures in the extract from eggs, tissue (or other media). The bioassays are an alternative to the TEF additive model derived from chemical analysis of individual congeners in a sample. It is also useful as a check (used in concert with chemical analysis) to determine whether all TEQs in a sample have been accounted for and whether non-additive interactions are occurring (Giesy *et al.,* 1994).

exhibit significant differences in breeding success between colonies where breeding sites have local differences in levels of contamination by organohalogen pollutants. Van den Berg et al., (1994) performed a study to determine if the low breeding success was related to contaminant levels. In this study, seventeen cormorant eggs were collected from two colonies, hatched in incubators, and the hatchlings were sacrificed at day one for observation of a number of biochemical and morphological parameters. Yolk sacs were also analysed for levels of dioxin.

Results showed large variations in dioxin levels among individual bird eggs from both colonies, but with all congeners measured in eggs at both sites having a 2,3,7,8-chlorine substitution pattern. However, despite the individual variation, there was a two to five fold differences in average PCB levels, and 25% difference in PCDDs and PCDFs levels in the yolk sac between the two colonies. When data was compared on an individual basis, significant concentration effects were observed for EROD induction, plasma free thyroxine reduction, yolk sac weight, relative liver weight and head size. For mono-ortho PCBs, EROD activity was elevated approximately five times, while plasma FT4 levels decreased by 50% in the concentration range 10-100 μg/g. As PCB concentrations increased within the range 10-100 μg/g lipid, yolk sac weight decreased by 50%, and liver weight increased 1.5-2%. For 2,3,7,8-substituted PCDDs and PCDFs, the head length of hatchlings decreased 15-20% within the concentration range 0.8-2 pg/g lipid. It was concluded that dioxins were probably playing a role in the observed low breeding success in cormorants (Van den Berg et al., 1994).

**Blue Heron**

A number of studies have been performed measuring chemical residues and associated biological effects in the Great Blue Heron GBH (*Ardea herodias*) living in contaminated regions in the Strait of Georgia, British Columbia, which are associated with pulp and paper mills. Birds living near water bodies in contaminated regions are exposed to chemical contaminants in their food and eat mainly fish, frogs, crustaceans and insects.

Four Great Blue Heron colonies in the Strait of Georgia, British Columbia [Crofton (pulp and paper mill), Vancouver (industrial), Nicomekl (agricultural), and Sidney Island ("reference")] were studied for the effects of contaminants on reproductive success (Elliott et al., 1988; 1989). In 1987, 57 active nests in Crofton failed to produce any successful nests. Many eggs were broken and destroyed during incubation and found on the ground below. Nest failure was associated with a threefold increase in TCDD levels in eggs compared to the previous year. The mean concentrations were 210 TCDD pg/g, wet weight, and 230 pg TEQ/g.

In other studies with GBH from British Columbia, Henshel et al., (1995) found the frequency of brain asymmetry in chicks was directly related to TCDD and TCDD-TEQ concentrations in eggs (range 0.46-8.81 pg TCDD/g and 0.99-14.63 pg TEQ/g, respectively). Body weight and yolk-free body weight of hatchlings also decreased with increasing TCDD concentration. Bellward et al., (1990) found EROD activity was highly correlated with TCDD concentration in eggs. Levels of activity in chicks from the most

contaminated site were 2.6 times greater than values in chicks from the least contaminated (reference) site.

Hart et al., (1991) examined growth and development in GBH chicks from British Columbia exposed in 1987 to PCDDs and PCDFs from a nearby pulp mill. They found plasma calcium concentration, yolk-free body weight, tibia length, wet, dry, and ash weights, beak length, kidney weight, and stomach weight, to be related to TCDD exposure (mean concentrations between 135 pg/g to 211 pg/g). Fewer down follicles were also observed on the heads of exposed chicks. Gross abnormalities observed in the chicks included subcutaneous edema of the neck, legs, and abdomen, and one case of a crossed bill.

Sanderson et al., (1994) found mean TEQ levels between 100 and 278 pg/g wet weight eggs from the Straight of Georgia in British Columbia were associated with gross abnormalities in GBH hatchlings, including subcutaneous edema, fluid in the brain cavity, a blocked cloaca causing intestinal uric acid accumulation, and an unresorbed yolk sac. TCDD concentration was found to be inversely related to body, yolk-free body, stomach, and intestine weight, tibia wet, dry, and ash weight, and tibia length. Hepatic EROD activity was also directly related to TCDD concentration.

Similar results have been reported in studies of exposed birds from other regions. For example, Thomas and Anthony (1999) found that the reproductive rate of GBH observed at sites near paper or pulp mills in Washington and Oregon was positively correlated with mean TCDD concentration in eggs, and individual fledge success was negatively correlated with TCDD concentration in eggs.

**Raptors**

Raptors are top predators in the food chain and consequently are at risk from exposure to dioxins through bioaccumulation of dioxins in their food. Poor breeding success in some bird populations has been linked to high concentrations of environmental pollutants containing organochlorine compounds, including dioxins. Consequently, a number of studies have been performed to establish a link between contamination with dioxins and biological affects in raptor species.

DDE, a metabolite of the pesticide DDT, has long been linked to poor breeding success in the bald eagle (*Haliaeetus leucocephalus*), and in other bird species, due to it causing egg shell thinning and consequent breakage of the eggs before hatching. The presence of PCBs in bald eagles has also been associated with poor reproductive success. For example, Bowerman et al., (2003) collected blood plasma from 309 nestling bald eagles from 10 sites in the Great Lakes between 1987 and 1992 to correlate chemical residues to breeding success. All breeding and productivity measurements were significantly inversely correlated with the geometric mean concentrations of PCBs and DDE in plasma. These measurements include productivity within sub populations, the ability to produce two or three young, and success rates in subpopulations.

In a study by Elliott et al., (1996), eggs of the bald eagle were collected during the breeding season from areas in British Columbia, Canada, within a gradient of exposure to chlorinated hydrocarbon pollutants originating from pulp mill points sources. Eggs were

incubated and hatched and the hatchlings sacrificed within 24 h for analysis of PCDD, PCDF and PCB concentrations and biochemical assay.

Residual yolk sacs of eggs collected near pulp mills contained higher concentrations of TCDD and PCDFs compared to reference areas, while no significant difference in PCB concentrations was found. However, there were no evident symptoms of TCDD-like toxicity in chicks, and no difference in laboratory hatching success between eggs from pulp mill sites and eggs from reference sites. Hepatic cytochrome P4501A (CYP1A) cross-reactive protein, indicative of exposure to TCDD, was induced nearly six-fold in chicks from near a paper mill compared to those from reference sites. Elevated enzyme activities in liver microsomes (EROD and BROD) were also significantly elevated in birds from contaminated sites. $TEQ_{who}$, TCDD and PCDF concentrations in yolk sacs were positively correlated with hepatic protein and elevated enzyme induction. The NOAEL, using hepatic CYP1A induction as a biomarker, was determined to be 100 pg TEQ/g egg (wet weight), and the LOAEL was 210 pg TEQ/g on a whole egg wet weight basis.

In a later study, Elliott and Norstrom (1998) determined the bald eagle breeding success in nine colonies on the coast of British Columbia from 1991-1995, including those near pulp mills, agriculture or industrial activity, by measuring nest success and comparing the results to levels of chlorinated hydrocarbons in eggs and in nestling plasma samples. The geometric mean of I-TEQs[13] in bald eagle plasma collected in sites along the coast ranged from 0.25 pg/g to 2.6 pg/g, wet weight. Mean concentrations of 2,3,7,8-substituted PCDDs, PCDFs, and I-TEQs in nestling plasma were highest in samples collected near pulp mills (Nanaimo and Crofton). Mean PCBs were highest in industrial sites, while organochlorine pesticides did not vary among sites. Productivity was high in most breeding areas and only weakly related to DDE concentrations in plasma. There was no relationship between individual nest productivity and I-TEQs level in eggs. In one study area (Crofton), average productivity (0.26 young/occupied territory) was found to be significantly lower in nine territories adjacent to a dioxin-fishery closure zone, than productivity (1.0 young/occupied territory) at eight territories outside of the closure area, although the specific cause of the low reproductive rate was not determined.

In another study with bald eagles, Anthony et al., (1999) collected eggs (N=25) during the summers of 1993 and 1994 from Adak, Tanga, Amchitka, and Kiska Islands, in the Aleutian Islands, Alaska, USA. Clutch size ranged from 1.6-2.7 (mean 2.2), with no consistent differences between islands or years. Nest success ranged from 48% (Kiska Island) to 86% (Amchitka Island). Productivity ranged from 0.65 young per occupied nest site (Kiska Island, 1993) to 1.24 young per occupied nest site (Amchitka island 1994). The low productivity on Kiska Island was associated with levels of DDE known to cause reproductive problems in bald eagles, and elevated levels of mercury. Eggshell thickness was also negatively correlated with PCB concentrations.

In a study with osprey in 1995 and 1996, osprey eggs (*Pandion haliaetus*) were exchanged between nests on the Wisconsin River, from an area downstream from bleached kraft-mill

[13] I-TEQs are the international TCDD-toxic equivalents based on I-TEFs proposed by Safe (1990), and Ahlborg *et al*., (1994).

facilities contaminated with TCDDs and PCDFs/PCDDs (Castle Rock and Petenwell Flowages), and two upstream reference sites (Rainbow Flowage and Mead Wildlife Area) (Woodford et al., 1998). Egg hatching and chick fledging rates were not significantly different for nests that had been switched compared to those which had not been manipulated. However, chicks that were switched to Castle Rock and Petenwell Flowages as eggs had a lower mass increase rate than chicks at upstream reference sites, despite greater levels of parental nest attentiveness and food provisioning at the contaminated site.

Elliott et al., (2001) collected osprey eggs from seven sites in British Columbia (1995-1996), Canada, within a gradient of exposure to chlorinated hydrocarbon pollutants originating from pulp mill points sources. Eggs were incubated and hatched and the hatchlings sacrificed within 24 hours for analysis of PCDD, PCDF and PCB concentrations and biochemical assay. Eggs collected downstream of a bleached-kraft pulp mill contained greater mean concentrations of TCDD (2,930 pg/g lipid) and PCBs than the reference sites located upstream. The mean concentration TEQs, calculated on a whole egg basis using $WHO_{98}$ avian TEFs, were 82 pg/g for the entire 38 hatched eggs, and 77 pg/g in the 13 unhatched eggs, and 126 pg/g for five eggs that hatched from the most contaminated site. Hatching success did not differ among sites. Biochemical parameters positively correlated with TEQs and PCBs included increased hepatic EROD activity, and higher tissue concentrations of some vitamin A compounds. No correlation was found for morphological and histological parameters such as edema, deformities, renal and hepatic porphyrin concentrations.

Kannan et al., (2003) measured PCDD, PCDF and PCB concentrations in archived livers of white-tailed sea eagles (*Haliaeetus albicilla*) collected in eastern Germany from 1979 to 1998 in order to characterise congener profiles, concentration trends over time, and to estimate TEQs (based on bird $WHO_{98}$ TEFs) for dioxin-like toxicity. They found concentrations in the range 4.2 to 342 pg PCDD/g, 3.3-651 pg PCDFs/g, and 64,000 to 89,400,000 pg PCBs/g wet wt in livers. PCB 126 accounted for 66% of total PCB-TEQs, while PCDDs and PCDFs accounted for 19%. They estimated a liver concentration NOAEL of 15,000 pg/g, wet weight, for the sea eagle based on CYP1A induction. Egg concentrations were not measured. TEQ concentrations in eggs are expected to be approximately 15 times lower than liver concentrations.

In another study, Koistenen et al., (1997) measured dioxin concentration in terms of toxic equivalents in extracts from Baltic white-tailed sea eagle tissue in Finland. TEQs measured by chemical analysis in sea eagles were 770 pg PCBs/g fresh weight and 270 pg PCDD/PCDF/g fresh weight. Toxic equivalents were also determined based on the H4IIE bioassay (TCDD-EQ) using extracts from eagle tissue. These were found to be 20% higher than measured TEQs, suggesting there were compounds in the extract contributing to dioxin-like toxicity, but not accounted for by measured TEQs.

**Tree Swallows**

Secord et al., (1999) examined the concentrations of dioxin-like PCB congeners in tree swallow (*Tachycineta bicolor*) eggs and nestlings from contaminated sites in the upper Hudson River, USA. Swallows were exposed to PCB in contaminated sediments through ingestion of emergent insects. Total PCB concentrations in surface sediments in the study area ranged between <100 and 1,400,00 ng/g. Concentrations in insects ranged from 573 ng/g to 11,700 ng/g. PCB concentrations in swallow eggs ranged from 5,940 to 29,500 ng/g, while concentrations in nestlings ranged between 721 and 62,200 ng/g (fresh wet weight). Tree swallow nestlings often were less contaminated than eggs from the same nest, presumably because net dietary accumulation was less than the accumulation of body mass in growing chicks. Growth dilution was not observed at sites where there was a high PCB content in the birds diet.

Toxic equivalent concentrations (TCDD TEQs) were calculated for dioxin-like PCBs, using bird-specific WHO TEFs, and these were in the range 410-25,400 pg/g (82-87% of TEQ derived from PCB 77). These TEQs are some of the highest values reported in the literature (Table 2.7) and greatly exceed values linked to adverse effects in a number of other bird species (Secord et al., 1999).

The breeding success of tree swallows in the region was studied over a two-year period (1994-1995). In 1994, reproductive success of swallows was impaired by high rates of egg mortality due to failure of eggs to develop and death of developed embryos, while there were no apparent effects on the growth and development of hatched nestlings in either year. In 1995, the number of fledglings and eggs produced was normal. In both years, a high frequency of abnormal behaviour was observed, such as aberrant nest building behaviour, abandonment of eggs, higher than normal clutch sizes, and burial of eggs in nest lining. Because tree swallows are migratory, and overwinter elsewhere, it was not possible to determine post fledgling survival (McCarty and Secord, 1999). The authors of the study concluded that tree swallows (and perhaps other passerines) are more resistant to the effects of PCBs than many other bird species. They noted that populations of more sensitive fish-eating birds were conspicuously absent from the area.

**Terns and Gulls**

The water birds inhabiting Green Bay, Lake Michigan, Wisconsin, exhibit reproductive failure and birth defects, which are believed to be associated with contamination by dioxins. Studies with Forster's tern (*Sterna fosteri*) from the early 1980s in Lake Michigan reported total mean TEQs 2,175 pg/g, wet weight, whole egg. The TEQs were derived for TCDD and PCBs using older TEF systems. At this concentration, the hatching success of terns was reduced by 50% in eggs collected and incubated in the laboratory (Elliott et al., 2001). At TEQs of 913 pg/g wasting in chicks began at day 17 after hatching, and by day 31 there was significant mortalities in chicks (Colburn and Smolen, 1996).

Tillitt et al., (1993) used the H4IIE rat hepatoma cell bioassay to determine TCDD-EQs in Foster's tern eggs collected from Green Bay, Lake Michigan (Great Lakes) in 1983, and in egg collected from a reference colony from Lake Poygan. The average TCDD-EQs at Green Bay were 214.5 pg/g and at Lake Poygan they were 23.4 pg/g. Based on results

from laboratory and field studies monitoring hatching success, deformity rates, and other biological parameters in the same colonies, the authors concluded that the TEQs were high enough to account for the reproductive failure in these birds. Similar results were found for Caspian tern eggs. TEQs were calculated based on different sets of TEFs (i.e. not $WHO_{98}$ TEFs as this study precedes these) and were found to vary by a factor of 40.

The potency of PCB-containing extracts from common tern (*Sterna hirundo)* eggs collected from Great Lakes colonies in 1986-87 were bioassayed by their ability to induce cytochrome P-450IA1-associated EROD activity in H4IIE rat hepatoma cells using TCDD as a standard (Tillitt et al*.,* 1991). TCDD-EQs for common tern egg extract from Lake Michigan and Lake Huron were 187.4 and 104.3 pg/g, respectively. Even though the biological significance of these values have not been established and may not be predictive of toxicological effects, when extracts from several species were considered together, the greatest values were found in areas with the most severely affected reproduction effects.

Dead black-backed herring gull chicks (N=37) were collected from a colony at Söderskär Game Research Station, in the central Gulf of Finland in 1991 (Hario et al*.,* 2000). Liver and leg muscle tissue was analysed for 23 PCB congeners. Of the PCB congeners, 28, 52, 118, 138, 153, made up >70% of total PCBs. Arithmetic mean (range) concentrations of the combined 6 congeners were 41.3 (8.2-81.0) µg/g wet weight in liver and 21.0 (6.3-65.4) µg/g in muscle. Congeners 118, 138, 153, 167, 170, and 180 were 2.7 times higher in liver than muscle. One outlying chick that died at hatching had 99 µg/g in liver and 9.8 µg/g in leg muscle; this data was not used in further analysis. All chicks found dead died of disease and showed signs such as inflammation of lungs and intestines, degeneration of the liver and cardial muscle, and kidney sepsis. In total, 8% of chicks were found diseased at 0-4 days old. Disease may be associated with high concentrations of PCBs found in liver and leg muscle of dead chicks (Hario et al*.,* 2000).

**Other birds**

White and Seginak (1994) studied the effects of dioxins on the nesting and reproduction of wood ducks (*Aix sponsa*) living in a 2,4,5-T herbicide contaminated wetland in Arkansas, USA. Residues in duck eggs, based on US $EPA_{1989}$ TEFs, ranged from 0.2 to 611 ppt wet weight. Egg TEQs were inversely correlated with hatching success. Egg TEQs in the range 20-50 pg/g wet weight egg (based on TCDD-TEQs for furans and dioxins, primarily PCDDs) resulted in a 30-40% drop in duckling production compared to ≤5 pg TEQ/g egg. A measurable reduction in egg hatchability was also evident in 5 to 20 pg TEQ/g egg; but these nests still produced nearly as many live ducklings as the ≤5 pg TEQ/g egg group. Failed eggs (n = 250) were examined and 20% of these were found to be desiccated, 45% were addled, and in a few full term embryos, lower bill deformities, and subcutaneous edema of the head and neck were observed. One deformed duckling was found to contain 32 ppt 2,3,7,8-TCDD and 19 ppt 2,3,7,8-ppt TCDF, with a whole body TEQ of 42 ppt. Based on these data, an $LD_{50}$ for hatching success would be approximately 70 pg TCDD-TEQ/g egg. Thus, the wood duck appears to be a relatively sensitive species to dioxin-like chemicals that bind to the Ah receptor.

Dioxins and other organochlorines are found in offshore marine birds in relatively remote areas away from pollution sources, attesting to the global scale of the contamination. Relatively high concentrations of PCBs have been found in fat samples from North Pacific populations of albatross (*Diomedea sp*), while lower concentrations were found in the South Pacific (Auman et al., 1997; Jones et al., 1996; Jones, 1999). The mean PCB concentrations in albatross eggs in the North Pacific are lower than those found in the eggs of piscivorous birds in the North American Great Lakes Region. Nevertheless, the TCDD toxic equivalent concentrations (Table 2.7) in black-footed albatross eggs are reported to be high enough to potentially cause subtle effects on reproduction based on observations in populations of fish eating birds of the Great lakes (Auman et al., 1997).

### 2.4.3 Summary of avian toxicity

Dioxins are highly toxic to birds in laboratory studies, with bird embryos exposed via maternal transfer being more sensitive than adults and hatchlings. Adverse effects in embryos following exposure to dioxins include embryo lethality, developmental abnormalities (deformities of the skull, beak, eyes and toes), changes in body and organ weights, and edema. Low breeding success and developmental abnormalities in wild bird populations in contaminated regions have been linked to the presence of Ah receptor agonists including dioxins.

In egg exposure studies, there is considerable variability among species in their sensitivity and response to dioxins. Galliformes are the most sensitive order of birds, with the domestic chicken the most sensitive species so far tested, while the eastern bluebird is the least sensitive. The LOAELs causing embryo mortality and developmental effects in laboratory studies are between 10 and 10,000 pg/g fresh weight TCDD in bird eggs for the most and least sensitive species, respectively. Other bird species shown to be less sensitive than chickens include ring-necked pheasant, mallard duck, domestic duck, domestic goose, double crested cormorants, blue heron, American Kestrel, ring-billed gull, herring gulls, Forster's tern, common tern, tree swallows, and tree swallow.

## 2.5 Mammalian Toxicity

The toxic responses to TCDD observed in mammals are varied and include mortality, reproductive and developmental effects, endocrine and immune system dysfunction, hemorrhaging, anemia, and wasting syndrome, and biochemical effects.

A large database is available for toxicity of TCDD to a limited number of laboratory mammals. The majority of these are performed predominantly with rats and mice exposed to dioxins in their food. There are also a significant number of *in vitro* studies exposing cultures and cells derived from the tissues of a limited number of species to TCDD. For the most part, these data are described in existing reports such as US EPA (2000d), and WHO (1989), and consequently will not be reviewed in detail in this report. Available toxicity data for mammalian wildlife are very limited.

### 2.5.1 Acute and chronic toxicity

There is significant variation among mammalian species in their sensitivity to the toxic effects of dioxins. In terms of acute toxicity following dietary exposure, the guinea pig is one of the most sensitive species tested so far, with an $LD_{50}$ for mortality of 600 to 2,100 pg/g (Table 2.8). Another sensitive species is mink (*Mustel vision*) with a 28-d $LD_{50}$ of 264 pg/g bw/day for the female mink and 4,200 pg/g/kg bw/day for the male mink (US EPA, 2000d). Rats appear to be the third most sensitive species after guinea pigs and mink. The least sensitive species appears to be the hamster with $LD_{50}$ of between 1,157 to 5,000,000 pg/g (US EPA, 1993; NLM Hazardous Substances Databank, 2002). These endpoints show a greater than 19,000 fold differences in sensitivity to TCDD following dietary exposure.

**Table 2.8 Comparative toxicity in mammals following dietary exposure to TCDD[14]**

| Species | LD50 pg/g bw/d |
|---|---|
| Hamster | 1,157,000-5,051,000 |
| Dog | 100,000-200,000 |
| Rabbit | 115,000 |
| Rabbit | 10,000 |
| Rhesus monkey (female) | <70,000 |
| Mouse (male) | 114,000 |
| Rat (male)<br>Rat (female) | 22,000<br>45,000 |
| Guinea pig (female) | 600-2,100 |
| Mink (male)<br>Mink (female) | 4,200<br>264 |

It is very difficult to compare sensitivities between mammalian species exposed to dioxins when toxicity data are based on dietary exposure because of widely differing exposure conditions, including dose level, dose media, duration of exposure, and lack of information on whether steady state concentrations were reached. The toxic effects caused by dioxins are a direct result of tissue concentrations reaching a particular threshold for a given response rather than the dose received. The key determinants in the tissue concentration reached by an organism are the rates of uptake, metabolism and excretion. Variability in these parameters among species results in large differences in the half-lives of dioxins across species (EU 2000, 2001). Consequently, the large differences in sensitivity in mammals following dietary exposure to dioxins reflect the differences in the doses required among species to reach the same equivalent body burdens.

### 2.5.2 Reproductive toxicity

[14] US EPA, 1993a; NLM 2003.

As with birds and fish, reproductive and developmental toxicity are among the most sensitive endpoints for mammals. A large number of developmental and reproductive studies have been performed with rats and mice, while a small number of studies have been performed with other animals, such as rabbits and monkeys. Most of these studies exposed adult females to TCDD in their diet, with the developing organisms being exposed through maternal transfer during gestation and lactation. A summary of some of the available data is provided in the Tables 2.9 and 2.10.

In the laboratory studies with mammals, the exposure conditions vary significantly and include exposure to a single dose, and to multiple doses administered over varying periods of time, ranging from weeks through to several generations. This makes it difficult to compare sensitivity to reproductive and developmental toxicity among species.

Dioxins are developmental toxicants at very low concentrations. When present from conception through postnatal stages, dioxins can disrupt the development of the endocrine, reproductive, immune and nervous system of the offspring. Developmental effects observed in laboratory studies include prenatal mortality, abnormal sexual development, and deformities such as cleft palate (Tables 2.9 and 2.10). In long-lived mammalian species with slow development times, many effects may not be readily apparent at birth, but may manifest long after the initial exposure as immune and reproductive dysfunction, abnormal development, and neurological and behavioural changes in later life (Colborn and Smolen, 1996).

The embryo, fetus and newborn are especially vulnerable to exposure through maternal transfer in the womb and after birth. It is thought that much of the developmental toxicity in young mammals is dependent on the time of exposure of the mother during pregnancy. Mammalian pregnancies are characterised by critical periods during which the embryo or fetus exhibit different susceptibilities and responses to chemical exposure at different developmental stages (US EPA, 2000d). The critical window may differ for different species, and therefore make it difficult to compare from the cumulative maternal dose. These factors further increase the difficulty making interspecies comparison of reproductive and developmental sensitivity of dioxins.

### 2.5.3 Toxicity studies with mink

Mink are one of the few wildlife species studied for their sensitivity to TCDD. These data are described in more detail below.

In one study with mink, sixteen adult mink were administered a single oral dose of TCDD in concentrations of 0, 2,500, 5,000, or 7,500 pg/g body weight and observed for 28 days (Hochstein et al., 1988). No animals died at the lowest doses; however, between 14 and 17 days post-exposure, 100 and 75% mortality was observed at doses of 7,500 and 5,000 pg/g body weight, respectively. A 28-day $LD_{50}$ value of 4,200 pg/g body weight was determined. Sublethal effects in mink receiving TCDD included reduced food consumption, body weight, and adipose tissue, gross necropsy revealed mottling and discoloration of the liver, spleen, and kidneys and enlarged brain, kidneys, heart, and thyroid and adrenal glands. Food consumption decreased significantly in the first week for

the 5,000 and 7,500 pg/g groups, increasing in the third week for survivors. Body weight was significantly lower in the survivors of the 2,500 pg/g group and individuals that died in the 5,000 and 7,500 pg/g groups. A significant increase in weight occurred in the kidneys and brains of 5,000 and 7,500 pg/g dosed mink, in the heart of the 5,000 pg/g animals, in the thyroid glands of the 7,500 pg/g group, and in the adrenal glands of all test groups.

**Table 2.9 TCDD acute developmental and reproductive toxicity in mammals following a single dose exposure [15]**

| Species | Effect | Dose | Gestation day | Effect level |
|---|---|---|---|---|
| Guinea pig | Prenatal mortality | 1,500 pg/g | 14 | LOAEL |
| Hamster | Prenatal mortality | 18,000 pg/g/day | 7 or 9 | LOAEL |
| Mouse | Prenatal mortality | 24,000 pg/g/day | 6 | LOAEL |
| Rat | Accelerated eye opening | 50 pg/g | 15 | LOAEL |
| Rat | Reduced ejaculated sperm numbers | 50 pg/g | 15 | LOAEL |
| Rat | Reduced ventral prostate | 64 pg/g | 15 | LOAEL |
| Rat | Reduced caudal epididymal sperm numbers | 64 pg/g | 15 | LOAEL |
| Rat | Vaginal thread malformation | 200 pg/g | 15 | LOAEL |
| Rat | Hypospadias (female) | 200 pg/g | 15 | LOAEL |
| Rat | Cleft phallus (female) | 800 pg/g | 15 | LOAEL |
| Rat | Partial feminisation of sexual behavior (male) | 160 pg/g | 15 | LOAEL |
| Rat | Demasculinisation of sexual behavior (male) | Single 64 pg/g | 15 | LOAEL |
| Hamster | Thymic hypoplasia | 1,500 pg/g | 7 or 9 | LOAEL |

[15] US EPA, 2000d, and references therein

**Table 2.10 TCDD chronic reproductive and developmental toxicity in mammals following multiple dose exposure**

| Species | Effect | Exposure | Dose | Gestation day | Effect level |
|---|---|---|---|---|---|
| Monkey | Prenatal mortality | Multiple dose | 22 pg/g, 9X | 20-40 | NOAEL |
| Monkey | Prenatal mortality | 0.642 pg/g/day<br>Multiple dose | 97 pg/g[a], subchronic 111 pg/g, 9X | 20-40 | LOAEL<br>LOAEL |
| Rat | Prenatal mortality | 1 pg/g/day | 27 pg/g[a], chronic | | NOAEL |
| Rat | Prenatal mortality | Multiple dose | 30 pg/g/day | 6-15 | NOAEL |
| Rat | Prenatal mortality | 10 pg/g/day<br>Multiple dose | 270 pg/g[a],<br>chronic 500 pg/g/day | 6-15 | LOAEL<br>LOAEL |
| Rabbit | Prenatal mortality | Multiple dose | 250 pg/g/day | 6-15 | LOAEL |
| Rat | Fetal growth | Multiple dose | 125 pg/g/day | 6-15 | LOAEL |
| Mouse | Hydronephrosis | Multiple dose | 100 pg/g/day | 6-15 | NOAEL |
| Mouse | Cleft palate | Multiple dose | 300 pg/g/day | 6-15 | NOAEL |
| Mouse | Cleft palate | Multiple dose | 3,000 pg/g/day | 6-15 | LOAEL |
| Mouse | Hydronephrosis | Multiple dose | 500 pg/g/day | | LOAEL |
| Rabbit | Extra ribs | Multiple dose | 100 pg/g/day | 6-15 | LOAEL |

[a]Maternal body burdens of TCDD at the time of conception were calculated by assuming a one-compartment open model and half-life for whole body TCDD elimination of 400 days in the monkey and 23.7 days in the rat. A bioavailabilty of 86.1 percent was used in the monkey and rat. The daily dietary exposure levels in rhesus monkeys were approxiately 5 and 25 ppt at the NOAEL and LOAEL doses, respectively. Rhesus monkeys were exposed to these levels of TCDD for 7 months prior to conception. At this time (0.525 half-lives) the cumulative amount of TCDD in rhesus monkeys was 30.5 percent of the calculated steady-state level. Rats were exposed to the indicated daily doses of TCDD for a period of 90 days (3.8 half-lives) prior to conception. At this time the cumulative amount of TCDD in rats was 92.8 percent of the calculated

No significant weight increase occurred in the liver or spleen, though gross necropsy revealed mottling and discoloration of these organs as well as the kidney.

Newborn mink are more sensitive to the toxic effects of TCDD than adults. Newborn mink were separated into six groups and administered daily 100 pg TCDD/g body weight, 1,000 pg TCDD/g body weight, 10,000 pg epidermal growth factor (EGF)/g body weight, 50,000 pg EGF/g body weight, a 1:9 acetone/corn oil mixture (TCDD vehicle), or 0.85% sodium chloride (EGF vehicle) for twelve consecutive days (Aulerich et al., 1988). Doses of 1,000 pg TCDD/g body weight were lethal to all test subjects within two weeks. Those treated with 100 pg TCDD/g body weight showed significant body weight reduction by two weeks for males and three weeks for females. Mortality in this group continued after dosing and was 62% by the 19$^{th}$ week. Significant weight reductions first occurred in both EGF groups in the second week in both males and females. Mortality occurred mainly during the dosing period in these groups. The number of days to eyelid opening was significantly reduced from a mean of 34.2 days in control to 27.4 days in the 10,000 pg EGF group and 15.1 days in the 50,000 pg EGF group. Eyelid opening was not significantly different in the TCDD groups, nor was incisor or premolar eruption for any of the test groups.

Mink are found to be very sensitive to reproductive toxicity of TCDD. Reproduction is disrupted by small amounts in the diet. Responses include reduced whelping, reduced litter size, and excessive kit mortality at birth and at 6 weeks of age (Leonards et al., 1995; Tillitt et al., 1996). Mink are almost entirely piscivorous and are therefore susceptible to exposure from eating contaminated fish.

In one study, sixty mink were fed diets consisting of 0, 10, 20, or 40% carp collected from Saginaw Bay, Michigan, prior to and throughout the reproductive period (Heaton et al., 1995). The resultant diets contained 0.015, 0.72, 1.53, and 2.56 mg PCB/kg diet or 1.03, 19.41, 40.02, and 80.76 pg TEQs/g diet, respectively. Food consumption was inversely proportional to percentage of carp in the diet. Mink fed the 40% diet exhibited signs of PCB intoxication including parental inattentiveness in females. The 40% group resulted in a significantly lower number of kits whelping, with all born stillborn or dying within 24 hours. Kit weight and survival to weaning was inversely proportional to percent of carp in diet. A dose-related decrease was measured in relative organ weights of the liver, spleen, kidney, lung, adrenal gland, and heart in six-week-old kits, with the 20% group showing significantly greater adrenal gland weights. Adult mink showed a general dose-dependent increase of all relative organ weights. Lowest observable adverse effect levels of 0.134 mg PCBs/kg body weight/day or 3.6 pg TEQs/g body weight/day for adult female mink were determined.

Leonards et al., (1995) reviewed existing studies with mink to determine an $LD_{50}$ for reproduction following exposure to mixtures of PCBs based on the experimental data. Because the conditions of the mink studies varied considerably, they used a bioaccumulation/biotransformation model to predict whole body concentrations in mink to correct for differences in exposure duration among the studies. They proposed critical body burden $EC_{50}$ values for mink litter size and kit survival, based on total PCBs in the adult female (wet weight), and based on TCDD equivalence/wet weight (using the TEF of non-ortho- and mono-ortho- substituted PCBs). As with most studies performed prior to 1998,

the authors used TEF systems, which predate the $WHO_{98}$ system (Table 2.11). The standard errors for the TEQ concentrations are 20 and 25% for litter size and kit survival, respectively.

**Table 2.11 Toxicity endpoints in mink based on tissue concentrations**

| Effect level | Exposure | Response | Reference |
|---|---|---|---|
| $EC_{50}$ | 1.2 µg/g PCBs/wet wt. muscle<br>40-60 µg/g PCBs/lipid weight (2-3% lipid)<br>160 pg/g TCDD equivalence/ww | Litter size | Leonards et al., (1995) |
| $EC_{50}$ | 2.4 µg/g PCBs/ww muscle<br>200 pg/g TCDD equivalence/ww | Kit survival | Leonards et al., (1995) |
| NOAEL | 11 pg TEQ/g liver weight<br><10 pg H4IIE TCDD-EQ/g liver weight | Reproductive effects | Tillitt et al., (1996) |
| LOAEL | 324 pg TEQ/g liver weight<br>495 pg H4IIE TCDD-EQ/g liver weight | Reproductive effects | Tillitt et al. (1996) |
| Threshold | 60 pg TEQ/g liver weight<br>70 H4IIE TCDD-EQ/g liver weight | Reproductive effects | Tillitt et al., (1996) |

In another study with mink by Tillitt et al., (1996), adult mink were fed a diet of PCDD, PCDF and PCB contaminated fish from Lake Huron for 52 days prior to breeding and 104 days prior to whelping, continuing until kits were approximately six weeks old. The study consisted of four treatment groups, with each group fed either 0 (control), 10, 20 or 40% contaminated fish. Reproductive toxicity was observed in all treatment groups fed carp. In the highest treatment groups, there was a significant decrease in the number of live kits whelped per female. At three weeks of age, a reduction in kit body weights occurred in the 10% carp and higher treatment groups, and at three to six weeks of age, there was a reduction in kit survival.

The LOAEL and the NOAEL for concentrations in the liver are shown in Table 2.11. The biomagnification factors from concentration in fish to concentration in liver were about 11 for TCDD for all treatment groups (i.e. varying dietary exposure concentrations), suggesting steady state was reached. An estimated threshold dose (ETD) was also calculated from the geometric mean of the NOAEL and the LOAEL. The TEQ concentrations in the diet and TEFs derived from the H4IIE bioassay were used to calculate total dioxin-like exposure. I-TEFs were used to calculate TEQs (Ahlborg et al., 1994). The authors indicated from limited data that TEQs derived from this bioassay are approximately 60% higher than the additive model TEQs estimated from concentrations measured in the same samples.

The mink dietary consumption to protect against reproductive effects was also determined in the study. A threshold daily food concentration of 0.42 pg TEQ per g of body weight per

day, and 0.96 pg H4IIE TCDD-EQ (g/bw/d) was estimated to be safe for mink reproduction. The Great Lakes Water Quality initiative estimates an NOAEL of 100 pg TCDD per g body weight per day to protect mink reproduction.

### 2.5.4 Epidemiological studies with wild mammals

A relatively large database exists of measured loads of persistent organic pollutants (POPs) in marine mammals from around the globe. Marine mammals accumulate high concentrations of bioaccumulating chemicals because of their high blubber content, which act as a reservoir for fat-soluble chemicals. Other reasons contributing to high loads in marine mammals include their relatively long life-span, their position as top predators in the food chain, and their limited capacity to metabolise and eliminate these chemicals (Tanabe et al., 1994).

Body burdens are highest in fish-eating species such as dolphins, and pilot and belugas whales. However, species such as baleen whales, which feed on phytoplankton low in the food web, also have the potential to bioaccumulate persistent organic pollutants (Colborn and Smolen, 1996). For male aquatic mammals, there is an increase in accumulation of persistent organic pollutants in the body with age, while for female mammals, there is a decrease after maturity. The decrease in the female body burden is due to the transfer of chemicals in lipid-rich milk to lactating offspring. It is estimated that female striped dolphins, for example, transfer over 60% of their PCB residues to the newborn calves in milk (Tanabe et al., 1994). This means newborn mammals are exposed to high levels of contaminants at critical periods of growth when endocrine, immune, and nervous systems are developing. The exposure levels during lactation are likely to exceed anything the mammals may encounter in the environment in later life (Colborn and Smolen, 1996).

High levels of contaminants, especially Ah-receptor active dioxins, have been linked to population instability, mass mortalities, major stranding episodes, reproductive impairment, endocrine and immune systems disturbance, and serious infectious disease in marine mammals. For example exposure to organochlorine contaminants is linked to high incidence of cancer in beluga whale (*Delphinapterus leucas*) populations resident in the Gulf of St. Lawrence on the US-Canada border (Martineau et al., 1987). High concentrations of PCBs have been implicated in causing disease and reduced reproductive capability in the Baltic grey seal (*Halichoerus grypus*), ringed seal (*Phoca hispida*), and Wadden Sea harbor seal (*Phoca vitulina*), and to viral infections and mass mortalities in Baikal seal (*Phoca sibrica*), and Mediterranean striped dolphin (*Stenella coeruleoalba*) (Colborn and Smolen, 1996, and references therein).

The link between the tissue levels of dioxins (and other chemicals) and the development of disease and mass mortalities is controversial because no laboratory dose-response relationships have been determined for marine mammals. In natural environments, there are always confounding factors present that limit the ability to make unequivocal firm conclusions about specific cause and effects relationships. These confounding factors include among others, the presence of natural toxins produced by marine algae and dinoflagellate, habitat disturbance, climatic factors, and simultaneous exposure to a range of chemicals (Kannan et al., 2000).

Relative to dioxins and furans, the contribution of coplanar PCBs to TEQs is found to be significantly higher in marine mammals than in terrestrial mammals (Tanabe et al., 1994). The minimum or lower range concentrations of PCBs found in the livers of diseased or dead marine mammals are in the range of 60,000 to 7,000,000 pg/g PCB on a wet weight basis (Kannan et al., 2000). Colborn and Smolen (1996) calculated that 9 of the 16 cetacean populations for which samples are available had body burden TEQs of 500 pg/g, striped dolphins were carrying 6,676 pg TEQ/g lipid, and harbour porpoises were carrying 526 pg TEQ/g lipid, at the time of sampling. These TEQ values are based on application of TEFs (Safe, 1990) for the small number of dioxin-like PCBs and, therefore, represent conservative estimates.

### 2.5.5 Semi-field studies with marine mammals

While no controlled laboratory studies have been performed with marine mammals, a number of feeding or semi-field toxicity studies are available, which provide evidence of the link between the toxicity and consumption of contaminated food containing PCBs and other organic pollutants.

In one study, a group of 12 captive harbor seals was fed wild-collected organochlorine-contaminated Baltic Sea (BS) herring for 93 weeks, while another group of 12 seals was fed less contaminated Atlantic Ocean (AO) fish. The estimated daily intake of TCDD equivalents (TEQs) by seals fed fish from the Baltic Sea was 288,000 pg TEQ/day, while seals fed fish from the Atlantic Ocean received about 29,000 pg TEQ/day. Seals fed BS fish accumulated 286 ± 17 pg TEQ/g lipid in the blubber, while seals fed AO fish accumulated 90 ± 6 pg TEQ/g lipid. Blood was sampled from both groups of seals every 6 to 9 weeks during the study for measurement of haemotological and immunological parameters. Seals from both groups remained healthy and exhibited normal growth patterns during the study. However, blood from the seals fed BS fish contained significantly less vitamin A, less natural killer cell activity (i.e. reduced immune function) and exhibited less lymphocyte proliferation following exposure to mitogens compared to the seals fed AO fish. The observed effects were attributed primarily to PCBs, which accounted for 80-93% of Ah-R active compounds (de Swart et al., 1994; Ross et al., 1994; Ross et al., 1996).

In a similar study with harbor seals, one group of ten seals was fed fish (plaice) from the Wadden Sea (WS) for a period of two years, and another group of ten seals was fed mackerel from the Atlantic Ocean. The concentrations of PCBs in fish were 0.2 and 0.1 µg/g, wet weight, respectively. The daily dietary exposures for seals fed different masses of fish were 1500 µg and 200 to 300 µg PCBs/seal/day, for WS and AO, respectively. Based on an average body weight for seals of 50 kg, the corresponding PCB intake by seals was estimated to be 30 µg PCBs/kg bw/day for the WS group, and 4 to 6 µg PCBs/kg bw/day for the AO group. During the study, one seal died, while the others appeared healthy. Blood samples from seals exposed to 30 µg PCBs/kg bw/day contained significantly lower retinol and thyroid hormone, and the reproductive success of the WS group was significantly lower than the AO group (Kannan et al., 2000, and references therein).

Based on the results of studies with harbor seals, the dietary NOAEL and LOAEL, and the maximum allowable toxicant concentration (MATC) of PCBs or TCDD TEQs in seal blood was determined (Table 2.2). Confounding factors in these studies include the lack of full dose-effect relationships from use of only two treatment levels, which results in over- and under-estimation of NOAEL and LOAEL, respectively. Other confounding factors include the differences in lipid content in the different fish species, and the potential influence of the different levels of nutrition between treatment groups, which were fed different amounts of fish (Kannan et al., 2000).

**Table 2.12 NOAELs and LOAELs for total PCBs and TEQs in seals**

| Exposure | NOAEL | LOAEL | Seal Group |
|---|---|---|---|
| PCB body weight/d | 5.7 µg/kg | 29.2 µg/kg | Seals fed herring from the Baltic Sea or the Atlantic Ocean |
| TCDD TEQ body weight/d | 0.58 pg/g | 5.76 pg/g | |
| TCDD TEQ lipid weight | 90 µg/kg | 286 µg/kg | |
| PCB wet weight fish | 100 µg/kg | 200 µg/kg | Seals fed plaice from the Wadden Sea or mackeral from the Atlantic Ocean |
| PCB body weight/d | 5 µg/kg | 30 µg/kg | |
| MATC<br>lipid weight in seal blood<br>wet weight in seal blood | <br>5,200 µg/kg<br>4.5 µg/kg | <br>25,000 µg/kg<br>16 µg/kg | |

In another study, blood was collected from five free ranging bottlenose dolphins from the central west coast of Florida and analysed for organochlorines including PCBs. Blood immunochemical parameters were determined *in vitro*, and results indicated a negative correlation between lymphocyte proliferative responses to mitogens and concentrations of PCBs (and DDT). Total PCB concentrations in blood ranged from 26,000 to 750,000 pg/g, wet weight (Lahvis et al., 1995). Based on the assumption that, the immunotoxic effects are primarily AhR mediated, an LOAEL for total PCBs in the blood of 26,000 pg/g was proposed by Kannan et al., (2000). However, the small samples size, absence of a reference population, and the presence of co-contaminants in the blood samples precluded the derivation of an accurate threshold dose.

Vitamin A (retinoid) is essential for normal growth and development, and provides resistance against microbial infection. Thus, hepatic retionoid measurements are suggested as sensitive indicators of PCB exposure (Murk et al., 1998). Semi-field studies examining hepatic retinoids and total PCB concentrations in naturally exposed feral and captive European otters (*Lutra lutra*), have shown that hepatic retinol and retinyl palmitate are significantly, negatively correlated with liver TEQs, calculated from the concentrations on non- and mono-*ortho* PCBs. In wild otters, concentration greater than 5 ng TEQ/g lipid, in the liver, are associated with a significant incidence of disease. From semi-field studies, $EC_1$ (1% of animals affected) and $EC_{90}$ (90% of animals affected) values of 1 ng and 5 ng TEQ/g lipid were determined, respectively. The NOAEL and LOAEL for hepatic retinoid concentrations were 1,000 pg TEQ/g lipid weight and 2,000 pg TEQ/g lipid, respectively.

An NOAEL for vitamin A deficiency of 4,000,000 pg PCB/g lipid for total PCBs in otter liver was also suggested (Murk et al., 1998).

### 2.5.6 Summary of mammalian toxicity

Dioxins are highly toxic to mammals and cause a cause a wide spectrum of adverse toxic effects including lethality, reproductive and developmental effects, endocrine and immune system dysfunction, hemorrhaging, anemia, wasting syndrome, and biochemical effects. Mammals vary significantly in their sensitivity. Mink and otter are among the most sensitive aquatic-feeding mammals, while Guinea pigs are among the most sensitive terrestrial-feeding mammals. As with birds and fish, reproductive and developmental toxicity are among the most sensitive endpoints for mammals.

In terms of acute toxicity following dietary exposure, the $LD_{50}$ for mortality in the guinea pig is 600 to 2,000 pg/g, the 28-d $LD_{50}$ for mortality of female mink is 264 pg/g bw/day and for males 4,200 pg/g bw/day. Newborn mammals are more sensitive to the toxic effects of TCDD than adults. For mink, doses of 100 pg TCDD/g body weight were lethal to >65% of young after 19 weeks.

For mink, an LOAEL and the NOAEL for reproductive effects was determined to be 324 pg and 11 pg TEQ/g liver weight. A threshold daily food concentration of 0.42 pg TEQ per g of body weight per day was estimated to be safe for mink reproduction. Based on the results of studies with harbor seals, a dietary NOAEL and LOAEL in seals of 90,00 ng and 286,000 ng TCDD TEQs/g, lipid, respectively, was determined.

## 2.6 Conclusion

Laboratory studies are available examining the toxicity of dioxins in a limited number of species of fish, birds, mammals, and invertebrates (Table 2.13). Very little data are available for wildlife, and no toxicity data are available for Australian native wildlife. Laboratory studies show that dioxins are aryl hydrocarbon receptor agonists, which cause a wide spectrum of adverse toxic effects in many vertebrate species, with embryos, fetus and newborn being especially vulnerable to exposure during gestation and lactation. Dioxins are particularly potent developmental toxicants at very low concentrations and can disrupt the development of the endocrine, reproductive, immune and nervous system of the offspring of fish, birds and mammals when exposed from conception through postnatal or post hatching stages.

Mammals, birds, and fish vary among species in their sensitivity to dioxins. The most sensitive fish species are the salmonids, while the most sensitive avian species belong to the order of galliformes. The most sensitive mammalian species tested so far are the mink and the Guinea pig. Aquatic algae and plants, invertebrates and amphibians are much more tolerant of TCDD than fish, birds and mammals. The observed lack of sensitivity of plants and invertebrates to TCDD is consistent with the view that the Ah receptor is not present in invertebrates or plants.

While many of the toxic effects of dioxins are dose-response related, the toxic effects following exposure occur as a function of body burden. The dose level and time it takes to reach the threshold concentration body burdens differ among species and depend on the amount of contaminated food consumed, and the rate of uptake, metabolism and elimination in the organisms.

**Table 2.13 Summary of available data and toxicity of TCDD**

| Organism | Studies | Toxic effects |
|---|---|---|
| **Fish** | Large number of studies with limited number of fish species (mainly freshwater) | Very highly toxic |
| **Birds** | Limited number of studies with restricted number of species | Very highly toxic |
| **Mammals** | Large number of studies with restricted number of species, mainly rats and mice | Very highly toxic |
| **Reptiles** | No data | Unknown |
| **Amphibians** | Limited number of studies with a few tadpole and frog species | Slightly toxic (100-1,000 fold less sensitive than to fish) |
| **Invertebrates** | Limited number of studies with freshwater and marine invertebrates (e.g. snail, Daphnia, benthic amphipod, sandworm, grass shrimp, clam, snail, annelid worm, mosquito larvae) | No toxic effects |
| **Plants** | Limited number of studies with aquatic plants i.e. duckweed, algae | No toxic effects |

# 3 Exposure Assessment

## 3.1 Introduction

This chapter assesses the nature and extent of exposure to dioxins experienced by Australian native fauna, discusses the main pathways of exposure in aquatic and terrestrial environments, and identifies the receptor species potentially exposed.

The exposure assessment relies predominantly on measured data collected during phase one of the National Dioxin Program (NDP), where a number of surveys were initiated to determine ambient levels of dioxin in the Australian environment. The surveys are the subject of separate reports (Müller et al., 2003a, Müller et al., 2003b, Correll et al., 2003, see Chapter 1 for details).

Bioaccumulation, through trophic transfer from soil and sediment is the main transport pathway governing the levels of dioxins in biota. Bioaccumulation is the term describing the process whereby there is an increase or enrichment over time in the concentration of a chemical in an organism relative to the concentration in the environment. Dioxins, furans and PCBs for which dioxin-like toxicity is established are lipophilic (fat loving), resistant to chemical and biological breakdown, and as such have a strong tendency to accumulate in fatty tissue.

Organisms are primarily exposed to dioxins through dietary intake of contaminated food or soil. When a prey organism containing a low concentration of dioxin is eaten, the predator's gastric juices extract fat from the organism into the gastrointestinal tract, from where dioxins are concentrated by the digestion process when fat is absorbed and metabolised. Once absorbed, dioxins are then stored in fatty tissue of the predator. Predators eat quite a number of prey organisms in order to meet their energy requirements which again adds to the amount they accumulate. This process is repeated each time contaminated food is eaten, such that dioxins gradually accumulate until a maximum or steady state (where uptake and elimination are equal) concentration is reached for a given environmental exposure level.

Dietary exposure to dioxins is determined from measured concentrations in food, in combination with information on the amount and composition of food in the target organism's diet. A further requirement to determine dietary exposure is toxicokinetic information on the rate of absorption, distribution, metabolism, and excretion from the organism. Toxicokinetic information is generally not available for wildlife.

The most appropriate measure of exposure for use in risk assessment of dioxins is not dietary exposure, but rather, the residue concentration in the tissue of the wildlife of concern. The tissue concentration, or body burden, represents the amount of dioxin that has accumulated in an organism over time from all exposure sources. The advantage of using body burden to assess exposure is that unknown or poorly understood factors, such as toxicokinetic information, the organism's dietary composition, and the route, frequency, duration, and conditions of exposure, can be largely ignored.

During the NDP survey, wildlife samples were collected opportunistically (i.e. no animals were sacrificed for the determination of dioxin body burdens). However, fewer animals were collected from some regions than was initially anticipated owing to a lack of availability of recently deceased animals. In many cases, the widespread drought conditions at the time of sample collection, resulted in the lipid content of fauna being too low for analysis, also excluding some species and individuals from analysis (Correll et al., 2003). This means that wildlife samples are not representative of all locations and species potentially exposed, and consequently, important components in systems or food chains have been missed. Other potential limitations in the data set arise because of sampling bias, a lack of replication, and failure to measure random variation in sample populations.

In addition to body burden and dietary exposure, a third measure of exposure to dioxins for use in risk assessment is the concentration in soil and sediment where the target organisms live and feed. Ultimately, dioxin loads in fauna depend on the levels in soils and sediments, from where they are transferred through food chains. Thus, the approach adopted in this assessment is to first determine exposure to dioxins from tissue concentrations measured in the NDP fauna survey, and then to further characterise exposure at locations where no fauna samples are available, using measured concentrations in soil and sediment to predict tissue concentrations of dioxins in organisms.

Toxic equivalence methodology is adopted to translate the complex mixtures of dioxin, furan and PCB congeners found in organism or abiotic media to a single exposure reference value, which is expressed in terms of Toxic Equivalents (TEQs). TEQs are calculated by multiplying the measured concentration of each congener in the tissue, soil or sediment sample by its congener-specific and species-specific $WHO_{98}$ TEF. The resulting values are added together to give a final TEQ in the organisms of concern that is comparable to the toxicity reference values (refer to Chapter 1 for more information on the TEF methodology).

In the NDP survey reports, the conventional approach of assuming half the limit of detection (LOD[16]) for non-detects was adopted to calculate TEQs in soil, sediment and fauna from the measured congener concentrations. In this exposure assessment, a more conservative (worst-case) approach is adopted whereby the full LOD for non-detects is used to calculate TEQs for risk assessment purposes. However, for most samples, there was very little difference in the TEQs calculated from the full and ½ LODs. Comparisons for birds are shown in Table 3.6. The average soil and sediment TEQs reported in the summary tables are cited directly from the survey reports, and are based on ½ LOD.

[16] The limit of detection (LOD) is method dependent and determined by the specific analytical equipment. The LOD is the amount that is statistically different from the blank samples. The LOD is usually set at 3 times the standard deviation greater than the blanks. This allows a confidence of >99.8% that a value measured above this point is not due to background noise. For dioxin analyses, the LOD is also sample and congener dependent. The magnitude of the LOD within a sample is influenced by the amount of baseline noise. A complex (noisy) baseline can result in difficulty in resolving peaks that identify individual congeners. The magnitude of the LOD between samples is influenced by the amount of lipid available for analysis.

## 3.2 Methods and approach

### 3.2.1 Defining bioaccumulation

Bioaccumulation is a complex and dynamic process involving interactions between the uptake of a chemical by an organism, and the losses of the chemical from the organism through elimination and metabolic transformation. Different terms are used in different disciplines and jurisdictions to describe this process. Concentration ratios (or enrichment factors) used as a measure of the amount accumulated in an organism relative to a particular exposure media are also expressed in different ways. The definitions used in this assessment are briefly reviewed below.

Bioconcentration refers specifically to the accumulation of a chemical from water in the tissue of an aquatic organism, particularly a fish, through uptake via the gills and skin when the only uptake mechanism into the organism is diffusion. Bioconcentration factors (BCF) are determined for different aquatic organisms by exposing them in laboratory experiments to a constant water concentration, and then comparing the chemical concentration in water to the concentration in the test organism's tissue after an equilibration period. The resulting BCF can be simply defined by the ratio:

$$BCF = C_{organism}/C_{ambient\ water}$$

For hydrophobic chemicals such as dioxins, reliable measurements for ambient water are not available, and hence, accumulation of chemicals by an organism cannot be referenced to a water concentration as required for a BCF. In any case, owing to dioxins poor water solubility uptake via the gastrointestinal tract is significantly more important than uptake from water via gills and skin, or uptake from respiration or direct contact (Gobas et al., 1993).

Bioaccumulation refers to the process of accumulation of a chemical in the tissue of organisms through any route. The bioaccumulation factor (BAF) is defined in the same way as the BCF, but because it represents the net accumulation of a chemical from all routes of exposure, it contains additional terms for dietary or other routes of exposure (e.g. respiration, direct contact). For dioxins, the BAF represent the relationship between the concentration in an organism relative to the concentration in its food:

$$BAF = C_{organism}/C_{food}$$

Biomagnification is the process by which chemicals entering the food chain are transferred through one or more trophic levels, and because of their resistance to breakdown, may increase in concentration systematically from one trophic level to the next. The biomagnification factor (BMF) describes the relationship between the chemical concentrations in an organism at one trophic level (T1, prey) to the concentrations in an organism at a higher trophic level (T2, predator):

$$BMF = C_{organism\ at\ T2}/C_{organism\ at\ T1}$$

For the purposes of this exposure assessment, the biota-sediment accumulation factor (BSAF) is the means by which soil and sediment exposure concentrations are related to concentrations in the tissue of organisms. The BSAF is defined as the ratio of the chemical concentration in sediment (or soil) compared to the concentration in an organism:

$$BSAF = C_{organism}/C_{sediment}$$

It is assumed that organisms accumulate highly lipophilic and poorly metabolised chemicals in proportion to their lipid content, all else being equal (US EPA, 2000e). Since organisms differ in their lipid content, bioaccumulation factors are usually expressed on a lipid-normalised basis. The BSAF is also normalised to organic carbon in the soil. This allows comparison between different organisms and sediment types having differing lipid and organic matter content.

Field-measured BSAFs represent long-term chronic exposure to dioxins and incorporate the net effects of all opposing and augmenting influences on bioavailability, accumulation, metabolism, biomagnification and the resultant body burden (McFarland, 1995). Species-specific BSAFs are applied to dioxin levels in soil and sediment to predict the range of exposures likely to be encountered by organisms in regions where fauna samples were not collected, but for which soil and sediment data are available. Biomagnification factors are not determined owing to the lack of measured tissue concentration in mid and low trophic level food species in the NDP survey data.

### 3.2.2 Determination of BSAFs

The most accurate approach to determine the BSAFs is from the measured chemical concentrations in an organism and in sediment samples collected at the same specific site where the organism resides. For sedentary or lower-trophic-level species, this is achieved relatively easily by analysing dioxin levels in co-located sediment and fauna samples. However, for free-ranging animals, particularly predatory birds, mammals and fish, which typically have large home ranges, a wide range of exposures could have been encountered, and hence, determining exposure levels is more difficult.

If a number of data points have been sampled within an animal's home range, exposure concentrations can be averaged throughout their range, although this approach might underestimate the contribution of contaminant hot spots to the animal's total loading, resulting in an overestimation of bioaccumulation factors (US EPA, 2000c). This situation is likely for urban/industrial areas given the sampling strategy adopted for data collection during the NDP survey was to avoid hot spots. The emphasis in sampling was to determine baseline dioxin levels, and as such, sites potentially subject to unusual local contamination were deliberately avoided during sample collection.

Another common approach to determine field exposure is to use the upper confidence limit of the range of exposures, although this might result in an underestimation of bioaccumulation factors as it may exaggerate the actual exposure (US EPA, 2000c).

The approach used in this assessment to calculate BSAFs for non-sedentary species (i.e. all except bivalves), is to compare congener concentrations in individual animals with the

average congener concentrations in soil and sediment samples collected as near as possible to the area where the animals were collected. Owing to the opportunistic nature of the NDP survey's fauna collection regime, however, many sampling locations were poorly matched to specific soil and sediment collection sites. In these instances, soil or sediment concentrations for each land use subcategory are averaged over an entire region or sub-region. The resulting congener-specific BSAFs are then used to predict congener concentrations in organisms from concentrations in soil and sediment.

## 3.3 Terrestrial exposure

Soil microorganisms, including bacteria, viruses, and fungi, due to their large biomass, are probably the important first links for transfer of organic chemicals from the soil into terrestrial food webs. Microbes consume organic matter in the soil, which is the substrate for absorption of persistent organic pollutants. Soil dwelling microbes are in turn consumed by small soil mesofauna (e.g. springtails, collembola, mites), although some soil-dwelling organisms, such as earthworms, may consume soil and organic matter directly. Herbivores may also be exposed to dioxins by directly consuming contaminated dust or soil along with grass and leaves. Low to mid-trophic level organisms (e.g. insectivorous birds) subsequently consume the soil-dwelling invertebrates (e.g. earthworms, insects), who are in turn eaten by higher organisms in the food chain, such as birds of prey. By this mechanism, absorbed dioxins move from the soil into the terrestrial food chain, exposing organisms not in direct contact with the soil.

### 3.3.1 Soils

Baseline dioxin levels were determined in 104 soil samples collected from 86 sites in priority air sheds and catchments (Müller et al., 2003a). The soil samples were collected from sites representing three broad geographical regions (Northern, South-Eastern, and South-Western Australia) and four land use types with exposure classifications of industrial, urban, agricultural and remote (Table 3.1). Further subdivisions were made of agricultural land-uses according to the predominant form of agricultural practice (grazing, cotton, vegetables, sugarcane, forestry, cereals).

**Table 3.1 Total PCDD, PCDF and PCBs, expressed as TEQ, in Australian soils**

| | | Geographic region | | | |
|---|---|---|---|---|---|
| **Landuse** | **pg/g dw** | **Northern** | **Southeastern** | **Southwestern** | **All** |
| Industrial (n = 27) | Range | 0.58 – 10.8 | 0.39 – 11.2 | 0.13 – 0.436 | 0.13 – 11 |
| | Median | 1.78 | 2.43 | 0.30 | 2.2 |
| | Mean | 3.46 | 3.42 | 0.29 | 3.1 |
| Urban (n = 26) | Range | 1.03 – 9.21 | 0.24 – 23 | 0.17 – 0.47 | 0.17 – 23 |
| | Median | 2.7 | 4.5 | 0.32 | 3.53 |
| | Mean | 4.1 | 6.7 | 0.32 | 5.6 |
| Agricultural (n = 23) | Range | 0.09 – 4.3 | 0.03 – 1.7 | 0.21 – 0.49 | 0.03 – 4.3 |
| | Median | 0.26 | 0.21 | 0.35 | 0.21 |
| | Mean | 1.16 | 0.40 | 0.35 | 0.54 |
| Remote (n = 28) | Range | 0.095 – 1.1 | 0.059 – 5.2 | 0.041 – 0.29 | 0.041 – 5.2 |
| | Median | 0.35 | 0.17 | 0.15 | 0.24 |
| | Mean | 0.47 | 0.66 | 0.16 | 0.51 |

Dioxins were found in all but one of the 104 Australian soil samples analysed (Müller et al., 2003 a). Only 15% of the samples exceeded 5 pg TEQ/g dw. In all regions combined, TEQs ranged between the limit of detection (0.05 pg TEQ/g dry weight, dw) and 23 pg TEQ/g dw (using mammalian TEFs and ½ LOD) (Muller et al., 2003 a).

Dioxin levels in soils from urban and industrial locations were higher relative to agricultural land-use and remote locations. The highest TEQ was found in an urban soil, was 23 pg/g dw found in urban soils in Wollongong and Hobart. In industrial areas, the highest TEQ values found were 11 pg/g dw, which occurred at three locations, Brisbane, Latrobe Valley, and Sydney. In agricultural soils, the highest TEQ was 4.3 pg/g dw, which occurred in a sugar cane soil from the Sunshine Coast. In remote soils, the highest TEQ value was 5.2 pg/g, which was found in the sample from the Royal National Park. This latter result is not surprising given that the Royal National Park is located between two large metropolitan centers, Sydney and Wollongong and has been subjected to numerous bushfires especially in recent times.

The higher chlorinated PCDDs/PCDFs dominate the congener profiles in soils, with OCDD overwhelmingly the most abundant congener, typically contributing between 60 to 99% of PCDD/Fs. This was true even for soils from remote locations such as the Simpson Desert (SA) and Coopers Creek (QLD), although elevated levels of OCDD were more pronounced in coastal environments. While OCDD was the dominant congener, it is the least toxic of the PCDD/F congeners treated in this report (Table 1.3), with a TEF of 0.0001, and therefore contributed less to the TEQ. On average, more than 80% of the toxic equivalency across soil samples is attributed to the 2,3,7,8-substituted PCDDs/PCDFs.

The dominance of higher chlorinated PCDD/F was less pronounced in more temperate regions of Tasmania, South Australia, and Western Australia (Müller et al., 2003 a). This is consistent with the global fractionation hypothesis of Wania and Mackay (1996), where the

less volatile and most persistent PCDDs accumulate in tropical environments, while the lower chlorinated PCDD/Fs are transported to colder climates.

Elevated levels of higher chlorinated PCDDs have previously been found along the entire coastline of Queensland (Gaus et al., 2001a, Prange et al., 2002). The source of the contamination along the coast is not known. A biogenic or geological source has been hypothesised because of a general lack of industrial sources in the region, and because of the distinctive congener profile, and the presence of similar contamination in marine sediment cores predating European settlement (Gaus et al., 2001a; Prange et al., 2002).

The dominant PCB congeners in all environments were PCB 118 (50-70%), PCB 105 (10-20%), and PCB 156 (average 10%), with soils from urban and industrial environments having the highest concentrations. There was no apparent difference in PCB congener profiles between different geographic regions (Müller et al., 2003).

### 3.3.2 Terrestrial mammals

The NDP fauna survey provided tissue concentrations for 53 terrestrial organisms, comprising 33 mammals, 19 birds and one reptile. The strategy employed in the fauna survey was to determine spatial variation in dioxin levels in a common native marsupial (i.e. kangaroo) from areas throughout the target regions. Besides the kangaroo, other marsupials sampled included a koala (*Phascolarctos cinereus*), possum (*Trichosurus vulpecula*) and bandicoot (*Isoodon macrourus*). No carnivorous marsupials were available. As a contrast, two dingo (*Canis familiaris dingo*) samples were included representing carnivorous placental mammals (Correll et al., 2003). Samples of the echidna (*Tachyglossus aculeatus*) and the platypus (*Ornithorhyncus anatinus*) were included to represent the monotremes. Both monotreme species are mid-trophic level animals that consume invertebrates, with the echidna representing the terrestrial environment and the platypus representing the (terrestrial) freshwater aquatic environment.

**Macropods**

Table 3.2 provides tissue concentrations measured in kangaroos and wallabies (*Macropus sp.*). The TEQs in kangaroos were generally low, although there was considerable variability among samples. TEQ ranged between 0.45 and 25.4 pg/g lipid (based on mammalian TEF and using the full LOD). The median and mean TEQs for the macropods were 0.87 pg/g lipid and 2.7 pg/g lipid, respectively. The highest TEQ was found in a composite sample of three macropod tails from Para Wirra, SA, conservation reserve (classified as remote), which is about 20 km north of Adelaide. The congeners contributing most to the TEQ in this sample were furans, in particular 2,3,4,7,8-PeCDF. The next most contaminated samples were from urban Perth, Jabiru in the NT, and two other samples from Para Wirra.

OCDD was the dominant PCDD/F congener in kangaroo tissue samples in terms of the relative concentrations, comprising about 78% of total PCDD/Fs. PCB 118 and PCB 105 were the dominant PCB congeners, comprising 50% and 20% of PCBs, respectively, with PCB 156 ranking third (13%). The dominant congeners in kangaroo tissue are the same as

those that dominate soils. On average, TEQs in kangaroos are not significantly higher than in soils.

Kangaroos are herbivores eating mainly grass, leaves and roots. Research indicates that dioxins are not readily translocated into the above ground foliage of plants but rather tend to bind to organic matter on roots. Dry or wet deposition of airborne, gaseous and particulate phase dioxins are considered the main pathways by which dioxins transfer to plants. The particle phase dioxins settle onto plants and are eventually washed or blown off from the plants, unless consumed by herbivores. Vapor phase dioxins "transfer" to plants through air-to-leaf biotransfer (Lorber, 1995). Thus, the principal route of exposure of adult and juvenile kangaroos to dioxins is expected to be through eating grass contaminated with soil particles containing dioxins. Joeys would be vulnerable to exposure from the maternal body burden during lactation.

**Table 3.2 TEQs in macropods**

| | | pg $TEQ_{/g}$ lipid | | |
|---|---|---|---|---|
| Species | **Sampling location** | **PCDD/F** | **PCB** | **Total** |
| Western grey kangaroo, *Macropus fuliginosus* | Para Wirra, SA | 17 | 8.4 | 25.4 |
| Western grey kangaroo, *Macropus fuliginosus* | Para Wirra, SA | 3.2 | 2.4 | 5.7 |
| Agile wallaby, *Macropus sp.* | Jabiru, NT | 2.5 | 2.9 | 5.4 |
| Western grey kangaroo, *Macropus fuliginosus* | Melville, Perth WA | 1.1 | 4 | 5.1 |
| Western grey kangaroo, *Macropus fuliginosus* | Para Wirra, SA | 1.8 | 2.5 | 4.3 |
| Western grey kangaroo, *Macropus fuliginosus* | Kalgoorlie, WA | 1.5 | 2.2 | 3.7 |
| Western grey kangaroo, *Macropus fuliginosus* | Kuipto Forest, SA | 1.8 | 0.96 | 2.8 |
| Wallaby/kangaroo, *Macropus sp.* | Healsville, VIC | 0.98 | 1.7 | 2.67 |
| Wallaby/kangaroo, *Macropus sp*. | Coolum/Noosa, QLD | 0.84 | 0.32 | 1.17 |
| Kangaroo, Macropus sp. | Amberly, QLD | 0.50 | 0.47 | 0.97 |
| Agile wallaby, Macropus sp. | Jabiru, NT | 0.36 | 0.53 | 0.90 |
| Western grey kangaroo, *Macropus fuliginosus* | Halls Creek, VIC | 0.64 | 0.2 | 0.84 |
| Eastern grey kangaroo, Macropus giganteus | Gunnedah, NSW | 0.23 | 0.60 | 0.81 |
| Eastern grey kangaroo, Macropus giganteus | Mansfield, VIC | 0.39 | 0.38 | 0.77 |
| Western grey kangaroo, *Macropus fuliginosus* | Dimboola, VIC | 0.37 | 0.35 | 0.72 |
| Western grey kangaroo, *Macropus fuliginosus* | Katanning, WA | 0.44 | 0.27 | 0.71 |
| Wallaby/kangaroo, *Macropus sp*. | Gladstone, QLD | 0.42 | 0.22 | 0.64 |
| Western grey kangaroo, Macropus fuliginosus | Mt Barker, WA | 0.27 | 0.23 | 0.50 |
| Agile wallaby, Macropus agilis | Jabiru, NT | 0.43 | 0.02 | 0.45 |
| Eastern grey kangaroo, Macropus giganteus | Springsure, QLD | 0.40 | 0.04 | 0.45 |
| Wallaby/kangaroo, *Macropus sp*. | Parndana, SA | 0.41 | 0.04 | 0.45 |
| Eastern grey kangaroo, Macropus giganteus | Tibooburra, NSW | 0.16 | 0.11 | 0.27 |

## Non-macropod marsupials

Table 3.3 provides tissue concentrations measured in non-macropod marsupials. In these marsupials, the highest TEQ was found in the possum (*Trichosurus vulpecula*) from Hanson Bay, Kangaroo Island, SA. This individual had significantly higher levels of 1,2,3,4,6,7,8-HpCDD than the koala (*Phascolarctos cinereus*) or the bandicoots. Similarly PCB 126 was over ten times higher in the possum than in the koala and the bandicoots. These differences in TEQs could be attributed to differences in the dietary composition of the different species and are not necessarily related to different environmental exposures in the areas of collection.

**Table 3.3 TEQs in other marsupials**

| | | pg $TEQ_f$/g lipid | | |
|---|---|---|---|---|
| **Species** | **Sampling location** | **PCDD/F** | **PCB** | **Total** |
| Brush tail possum, *Trichosurus vulpecula* | Hanson Bay, SA | 3.2 | 10.3 | 13.5 |
| Northern Brown bandicoot, *Isoodon macrourus* | Jabiru, NT | 1.4 | 0.70 | 2.1 |
| Northern Brown bandicoot, *Isoodon macrourus* | Jabiru, NT | 1.8 | 0.14 | 1.95 |
| Koala, *Phascolarctos cinereus* | Crafers, SA | 0.83 | 0.62 | 1.45 |

The possums' diet consists of a variety of foods including leaves, fruit, buds, sap and bark. Many possums have adapted to the urban environment and consume practically any food that is available. Possums frequently raid garbage bins and may consume meat if it is available. Koalas consume only eucalyptus leaves.

## Monotremes

Table 3.4 provides tissue concentrations in monotremes. TEQs in monotremes range between 9.7 for a platypus (*Ornithorhyncus anatinus*) and 60 for an echidna (*Tachyglossus aculeatus*) from Port Elliot, SA.

**Table 3.4 TEQs in monotremes**

| | | pg $TEQ_f$/g lipid | | |
|---|---|---|---|---|
| **Species** | **Sampling location** | **PCDD/F** | **PCB** | **Total** |
| Echidna, *Tachyglossus aculeatus* | Port Elliot, SA | 53 | 7 | 60 |
| Echidna, *Tachyglossus aculeatus* | Kersbrook, SA | 37 | 4.3 | 41.1 |
| Echidna, *Tachyglossus aculeatus* | Cleveland, TAS | 20 | 3.4 | 23.6 |
| Platypus, *Ornithorhyncus anatinus* | Strathgordon, TAS | 17 | 13.6 | 31 |
| Platypus, *Ornithorhyncus anatinus* | Glengarry, TAS | 3.9 | 5.7 | 9.7 |

The dominant congener in echidnas was OCDD (TEF = 0.0001), which contributed between 65 and 93% of PCDD/F in terms of overall concentration. The maximum OCDD concentration (14000 pg/g) was found in the echidna sample from Port Elliot. The second most dominant congener was 1,2,3,4,6,7,8-HpCDD, contributing from 6 to 18%. Much of the TEQ contributions were from PCDD congeners, and in particular 1,2,3,7,8-PeCDD, for which the TEF is equal to one. The dominant PCB congeners were PCB 118 (39-50% total PCB), PCB 156 and PCB 105.

The dominant congener in the platypus samples from Tasmania was also OCDD, accounting for 35 to 37%. Other PCDD/F congeners also present in significant amounts were 1,2,3,4,7,8 HxCDD and 1,2,3,6,7,8 HxCDD. However, PCBs contributed a much higher proportion of the TEQs in the two platypus compared to the echidnas. The dominant PCB congeners were PCB 118 (32 to 40% total PCBs), PCB 156 (21 to 30% total PCBs) and PCB 167 (19% total). The highest PCB concentration was 4500 pg/g for PCB 118 in one individual.

The monotremes had significantly higher TEQs than the herbivorous marsupials. Echidnas are mid-trophic-level feeders, with a diet made up primarily of ants and termites. Secondary food items include grubs, larvae and worms. These latter organisms are known to accumulate dioxins. Echidnas use their sticky tongue to collect ants and termites from their nests, and in the process consume large quantities of soil and ant-nest material. The high level of OCDD and higher TEQs in the echidnas may therefore result from their consumption of soil and soil-dwelling invertebrates, although the number of samples is too small to make any firm conclusions.

The platypus is an aquatic mammal, and also mid-trophic level feeder. The platypus diet comprises worms, insects, crustaceans, molluscs, small vertebrates including tadpoles, and insect larvae. They live in burrows in the banks of rivers and streams, and spend much time grooming their fur. Consequently, the platypus is likely to consume large amounts of soil and sediment when foraging, digging and grooming. The reason for the higher contributions of PCBs to TEQs in the platypus is unclear. The platypus samples were collected from different locations, classified as remote (Strathgordon) and agricultural (Glengarry). Strathgordon is the location of the Gordon River hydroelectricity scheme.

**Dingo**

Only two dingo (*Canis familiaris dingo*) samples were found, one adult and one immature dingo. Both animals were collected from remote pastoral country. Table 3.5 provides tissue concentrations measured in these dingo samples. Two goannas were also submitted for analysis, however, only a Heath goanna (*Varanus rosenbergi)* from Penneshaw on Kangaroo Island, SA, had sufficient lipid for analysis (Correll et al., 2003).

Both dingo samples had very low concentrations of dioxins. PCDD/Fs contributed the highest to TEQ values. The TEQ for goanna was also very low, at only 0.89 pg TEQ/g lipid. The number of samples is too small to determine whether these low values are typical of dingoes and reptiles.

**Table 3.5 TEQs in dingo and a goanna**

| | | pg TEQ/g lipid | | |
|---|---|---|---|---|
| **Species** | **Sampling location** | **PCDD/F** | **PCB** | **Total** |
| Dingo, *Canis familiaris* | Ceduna, SA | 2.8 | 0.09 | 2.89 |
| Dingo, *Canis familiaris* | Ceduna, SA | 2.4 | 0.13 | 2.53 |
| Heath goanna, *Varanus rosenbergi* | Penneshaw, SA | 0.51 | 0.38 | 0.89 |

Dingoes are high trophic level carnivores. The most common elements in their diet are the macropod marsupials, which comprise around 40 to 70% of the diet (Robertshaw and Harden 1985, 1986; Newsome et al., 1983a, 1983b; Whitehouse 1977). However, the dingo will switch prey when availability of primary prey changes (Corbett and Newsome 1987). Dingoes in arid central Australia eat predominantly lizards, rabbits, and rodents. Dingoes in tropical northern Australia prefer magpie geese, rats, and small wallabies.

### 3.3.4 Avian wildlife

Dioxin levels in 19 avian species from different trophic levels are available from the NDP survey data. Bird species included the herbivorous galah (*Cacatua roseicapilla*), the pheasant coucal (*Centropus phasianinus*), which is a mid-trophic level feeder consuming mainly invertebrates and small reptiles, and birds of prey, which are at the top of the food chain. All of the birds of prey belong to a single taxonomic order, the falconiformes, covering 2 taxonomic families, the *falconidae* and *accipitridae*. The dioxin levels measured in birds are summarised in Table 3.6.

The TEQs in birds (using avian TEFs and including the full LOD) range between 1.0 pg TEQ per g lipid in the galah to over 3506 pg TEQ per g lipid in a collared sparrowhawk. The sparrowhawk was collected in an urban environment in Adelaide. The second highest TEQ was found in a female kestrel, also from Adelaide. The third highest TEQ was found in a sparrowhawk from urban Perth, while the fourth highest TEQ was found in a peregrine falcon, also from urban Adelaide. PCBs contributed the majority of TEQ in the birds from Adelaide, amounting to 76%, 91% and 66%, respectively from highest to lowest TEQ. PCDDs contributed the highest (92%) to TEQ in the bird from Perth.

The pheasant coucal had a low to mid-range TEQ of 76.8 pg/g lipid. The lowest TEQ measured in a bird of prey was 9.3 pg TEQ/g lipid for a Black-shouldered kite from Bremer River, SA. For all birds of prey, the average TEQ was 780 pg TEQ/g lipid and the median was 337 pg TEQ/g lipid.

The dominant PCDD/F congeners in bird tissue, averaged over all samples, were OCDD (36%), 1,2,3,6,7,8-HxCDD (24%), 1,2,3,7,8-PeCDD (12%), 1,2,3,4,7,8-HxCDD (6%), TCDD (5%), and 2,3,4,7,8-PeCDF (5%). For PCBs, the dominant congeners were PCB 118 (48% total PCBs), PCB 167 (21%), PCB 156 (13%) and PCB 105 (11%). These generally reflect the dominance pattern congener profiles in soils, although the TEQs in birds are significantly higher than the TEQs in soils.

**Table 3.6 TEQs in avian wildlife calculated from the full LOD[17]**

| | | pg TEQ/g lipid | | |
|---|---|---|---|---|
| **Species** | **Sampling location** | **PCDD/F** | **PCB** | **Total** |
| Collared sparrowhawk, *Accipiter cirrhocephalus* | Adelaide, SA (U) | 807.1 | 2684.1 | 3506.2 (3491.2) |
| Kestrel female, *Falco cenchriodes* | Adelaide, SA (U) | 192.2 | 1931.4 | 2123.8 (2123.6) |
| Sparrowhawk, *Accipiter cirrhocephalus* | Jane Brook, Perth WA (U) | 1701.9 | 145.3 | 1847.4 (1847.3) |
| Peregrine falcon, *Falco peregrinus* | Adelaide, SA (U) | 525.2 | 1020.7 | 1546 (1446) |
| Sparrowhawk, *Accipiter cirrhocephalus* | Beldon, Perth, WA (U) | 283.4 | 711.6 | 995.2 (995) |
| Black-shouldered kite, *Elanus axillaris* | Elizabeth, SA (U) | 624.2 | 181.4 | 805.6 (805.6) |
| Black-shouldered kite, *Elanus axillaris* | Penola, SA (A) | 83.7 | 576.0 | 660 (660) |
| Collared sparrowhawk, *Accipiter cirrhocephalus* | Penola, SA (A) | 259.2 | 218.5 | 479.7 (478) |
| Peregrine falcon, *Falco peregrinus* | Pt Lincoln, SA (A) | 225.0 | 112.0 | 337 (337) |
| Eagle-breast, *Aquila audax* | Woodford, QLD (A) | 118.9 | 140.2 | 259.4 (259.1) |
| Eagle-liver, *Aquila audax* | Woodford, QLD (A) | 138.6 | 64.2 | 204.5 (202.8) |
| Hobby falcon, *Falco longipennis* | Balga, Perth, WA (U) | 22.0 | 100.4 | 122.4 (122) |
| Kestrel male, *Falco cenchriodes* | Adelaide, SA (U) | 41.3 | 76.4 | 117.7 (117.7) |
| Brown goshawk, *Accipiter fasciatus* | Penola, SA (A) | 66.9 | 47.1 | 114.5 (114) |
| Brown goshawk (northern), *Accipiter fasciatus* | Gympie, QLD (A) | 75.8 | 14.6 | 90.7 (90.5) |
| Pheasant coucal, *Centropus phasianinus* | Darwin, NT (U) | 53.2 | 23.2 | 76.8 (76.4) |
| Brown falcon, *Falco berigora* | Darwin, NT (U) | 46.6 | 4.1 | 50.8 (50.7) |
| Black-shouldered kite, *Elanus axillaris* | Bremer River, SA (R) | 4.2 | 5.1 | 9.3 (9.3) |
| Galah, *Cacatua roseicapilla* | Darwin, NT (U) | 0.6 | 0.4 | 1.0 (0.6) |

[17] TEQs calculated from ½ LOD are provided in brackets underneath (U = urban, R = remote and A = agricultural

Differences in the amount of dioxins accumulated in birds of different trophic levels relative to the levels in soils can be explained in part by the difference in their diet. Table 3.7 provides a summary of the main dietary components as well as the average body weight and the main type of regular movements of the bird species sampled in the NDP survey.

**Table 3.7 Body weight, diet, movement and preferred habitat of avian species sampled in the NDP survey**

| Species | Body weight g | | Main prey | Habitat/movement |
|---|---|---|---|---|
| | Female | Male | | |
| Brown falcon, *Falco berigora* | 680 | 500 | Insects, reptiles, birds, mammals, generalist | Woodland, forest. Mainly resident, partially migratory inland. |
| Hobby falcon, *Falco longipennis* | 290 | 210 | Large Insects, small birds | Woodland, forest. Partially migratory. |
| Peregine falcon, *Falco peregrinus* | 920 | 610 | Mainly birds | Woodland, forest. Resident. |
| Australian kestrel, *Falco cenchriodes* | 185 | 165 | Insects, small birds, rodents | Grassland and open woodland. Partially migratory. |
| Collared sparrowhawk, *Accipiter cirrhocephalus* | 225 | 125 | Birds (small passerines), insects, lizards | Woodland, forest, grassland. Partially migratory, resident. |
| Brown goshawk (northern), *Accipiter fasciatus* | 360 | 220 | Birds, insects, reptiles, mammals | Woodland, forest. Resident to partially migratory. |
| Brown goshawk, *Accipiter fasciatus* | 565 | 340 | Birds, insects, reptiles, mammals | Woodland, forest. Resident, partially migratory. |
| Wedge-tailed eagle, *Aquila audax* | 3950 | 3100 | Mammals, reptiles, birds, occasional carrion | Open woodland, grassland. Resident. |
| Black-shouldered kite, *Elanus axillaris* | 300 | 260 | Small rodents, insects | Grassland. Resident. Eruptive, local movements. |
| Galah, *Cacatua roseicapilla* | 330 | 330 | Seeds, grasses, herbs, roots | Open woodland, grassland. Breeding pairs resident. Non-breeding birds may travel large distances (>1000 km) to feed. |
| Pheasant coucal, *Centropus phasianinus* | 440 | 300 | Large insects, frogs, lizards, eggs, young birds, small mammals | Open forest, woodland. Resident. Often lives in sugarcane, and dense undergrowth |

Galahs, with the lowest TEQ, consume mainly plant material, and therefore, would not be exposed through biomagnification to the same levels as high trophic level consumers. While galahs may consume some soil in their diet, there is a tendency for the bioavailability of dioxins to be reduced when they are absorbed onto mineral surfaces and organic matter, compared to dioxins dissolved in lipids of prey. Pheasant coucal consume mainly frogs, reptiles and insects and are likely to be exposed through biomagnification to higher doses than the galah. However, because of the small number of samples of low and mid- trophic-level birds, no firm conclusions can be made regarding the influence of trophic level on body burden.

The differences in dioxin loads between individual birds of prey from the same trophic level are likely to result from differences in exposure levels encountered among individual birds due to differences in habitat and diet. Exposure levels in soils are higher in urban and industrial environments, resulting in higher exposure in the prey items of urban birds. Other factors influencing body burden could include differences in age, gender, and movement patterns (Table 3.7).

Raptors are relatively long-lived birds. Ages reported for wild banded and captive birds range between 12 to 40 years. This means that these species integrate exposures over relatively long time frames, and hence, the tissue concentrations of older birds are expected to represent steady-state conditions. Younger animals may have initially high body burdens derived from maternal transfer than older animals given the same environmental exposure levels, however, as animals grow, and their body size increases, overall concentrations in the organisms may initially decrease by growth dilution. Adult females may also have lower levels than adult males due to the removal of some of their body burden when they lay eggs.

Raptors require a daily maintenance diet of 5 to 25% of their body weight, but this is greater for smaller birds (e.g. Sparrowhawk ~ 30%). Female raptors are generally larger than the males and may require more food, all else being equal. In cooler weather, and during breeding, birds may require double the maintenance diet.

Movement patterns of raptors vary from resident to partially migratory. Free-ranging predatory birds with large home ranges may encounter a wide range of exposure levels. They might also have been in the sampling area for a considerable length of time or only briefly, and hence, the duration of exposure becomes an unknown variable. The resident species tend to remain in or near their breeding grounds all year, although some individuals may move over long distances. The approximate home range for breeding pairs of raptors is probably in the order of 10 to 200 km² for most species (Olsen, 1995). Partially migratory birds may migrate when food is scarce or in winter when they move north to warmer climates.

## 3.4 Biota-soil accumulation factors for terrestrial fauna

The BSAFs for terrestrial and aquatic biota are presented in graphical form in the relevant sections that follow. The data have been normalised to lipid and organic carbon in soils.

The numbers on the x-axis represent the 29 dioxin-like congeners, numbered 1 to 29[18]. The Y-axis represents the ratio of the congener concentration in the organism divided by the soil/sediment concentration. The BSAFs are expressed in scientific notation and on a log scale, where 1.00+00 = 1, 1.00+01 = 10, 1.00+02 = 100, and so on. However, the ratios themselves have not been log transformed.

A BSAF equal to one indicates that the congener concentration is the same in the soil as in the organism (on a lipid normalised basis), a BSAF less than one indicates the congener concentration is higher in the soil than in the organism, and a BSAF greater than one indicates the congener concentration is higher in the organism than in the soil. The latter case implies that bioaccumulation has occurred.

Theoretically, if a congener in an organism is at equilibrium with the congener in the exposure media, the BSAF is expected to equal the ratio of the lipid-normalised partition coefficient ($K_{ow}$) for a congener to the organic-carbon normalised partition coefficient for the soil or sediment ($K_{oc}$). When dioxin congeners partition between two similar organic phases (organic carbon in soil and lipid in organisms), the expected equilibrium BSAF is unity or slightly greater. This is because $K_{oc}$ is of similar magnitude to and varies proportionally with $K_{ow}$ (US EPA, 1993a).

In reality, disequilibrium (resulting in lower or higher BSAFs) between concentrations in sediment/soil and organisms is frequently observed. This is thought to occur due to kinetic limitations for chemical transfer from sediment/soil to intermediate compartments such as the soil/sediment pore water, resulting in surface-sediment concentrations having not reached steady state. Additional factors that could cause disequilibrium include organic carbon breakdown during sediment turnover, and biological processes such as biotransformation or biomagnification. For example, steady state BAFs and BMFs can greatly exceed the equilibrium expected based on sediment, soil, water and food concentrations, because the removal of lipids and other organic material from food during digestion increases the activity of the chemical in the gut and promotes uptake (US EPA, 1993a).

[18] 1= 2,3,7,8–TCDD, 2 = 1,2,3,7,8–PnCDD, 3 = 1,2,3,4,7,8–HxCDD, 4 = 1,2,3,6,7,8–HxCDD, 5 = 1,2,3,7,8,9–HxCDD, 6 = 1,2,3,4,6,7,8–HpCDD, 7 = OCDD, 8 = 2,3,7,8–TCDF, 9 = 1,2,3,7,8–PnCDF, 10 = 2,3,4,7,8–PnCDF, 11 = 1,2,3,4,7,8–HxCDF, 12 = 1,2,3,6,7,8–HxCDF, 13 = 1,2,3,7,8,9–HxCDF, 14 = 2,3,4,6,7,8–HxCDF, 15 = 1,2,3,4,6,7,8–HpCDF, 16 = 1,2,3,4,7,8,9–HpCDF, 17 = OCDF, 18 = PCB 77, 19 = PCB 81, 20 = PCB 126, 21 = PCB 169, 22 = PCB 105, 23 = PCB 114, 24 = PCB 118, 25 = PCB 123, 26 = PCB 156, 27 = PCB 157, 28 = PCB 167, 29 = PCB 189.

### 3.4.1 BSAFs for marsupials

Figure 3.1 is a plot of the average BSAFs for 29 dioxin-like congeners determined for kangaroos/wallabies, a koala, a possum and two bandicoots. The concentrations ratios in kangaroos/wallabies were calculated using the average concentrations in kangaroos (n = 22) and soils from around Australia (n = 104), as these samples were collected from the target region. No attempt was made to match kangaroo sampling locations with soil sampling locations. The BSAFs for other marsupials were determined using the average congener concentrations in soils as near as possible to the region where the fauna was collected.

BSAFs for marsupials differ for each congener, but follow a similar pattern. In general BSAFs decrease for both PCDDs and PCDFs with increasing chlorination, with higher chlorinated compounds bioaccumulating the least. The dioxin and furan congeners with the highest bioaccumulation potential include those with 4 to 6 chlorine atoms i.e. 1,2,3,7,8-PeCDD, 2,3,7,8-TCDD, 1,2,3,4,7,8-HxCDD, 1,2,3,6,7,8-HxCDD, and their PCDF counterparts. This pattern is less clear-cut in the case of the koala and the possum, for which only one sample each was available.

For kangaroos and bandicoots, BSAFs of all PCDD/F congeners are less than one, indicating bioaccumulation is not occurring relative to soil concentrations. The BSAFs for PCBs are also less than one for kangaroos, but are between one and two orders of magnitude higher in the possum collected at Hanson Bay on Kangaroo Island than in soil, with PCB 81, 126 and 169 having the highest BSAF.

### 3.4.2 BSAFs for monotremes

Figure 3.2 shows the BSAFs of each of the 29 dioxin-like congeners in platypus (two samples) and in the echidna (three samples).

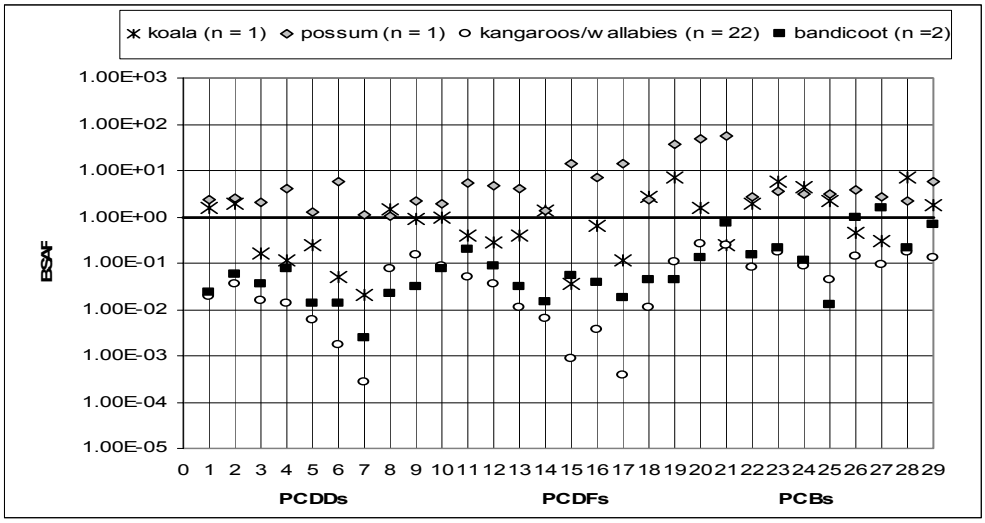



**Figure 3.1 BSAFs of PCDDs (1-7), PCDFs (8-17) and PCBs (18-29) for various marsupial species**

The BSAFs for platypus were calculated using the average concentration in Tasmanian freshwater sediments. However, these sediments were not collected at the same location as the platypus, for which no sediment samples were available. The BSAFs for the echidnas were calculated using the average concentrations in soils from the states where they were collected. The BSAFs are higher in echidnas than in platypus. Dioxins, furans and PCBs occur at elevated levels in the echidnas compared to soils.

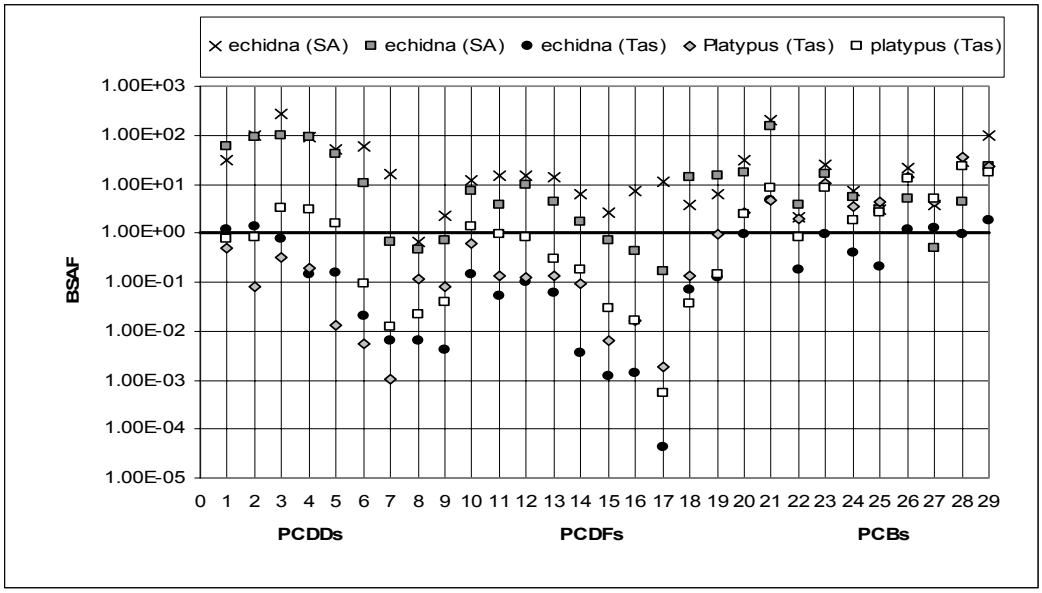



**Figure 3.2 BSAFs of PCDDs (1-7), PCDFs (8-17) and PCBs (18-29) for monotremes**

### 3.4.3 BSAFs for birds

Figure 3.3 shows the BSAFs for the 29 dioxin-like congeners in birds from different trophic levels, including the two non-raptors. The BSAFs were calculated from the congener concentration in each bird relative to the average levels in soil samples collected within the same broad region where the birds were collected. If more than one sample of the same bird species was available, the BSAF data were averaged.

The highest BSAFs occur in the sparrowhawks from WA and SA (n = 2 each) and the peregrine falcons from SA (n = 2). The BSAF for the galah, a herbivore, from the NT is overall much lower than for the other bird species, varying by about two to three orders of magnitude (depending on the congener) between the galah and the raptors. The BSAFs for the pheasant coucal, a mid-trophic level feeder, fall in between the raptors and the galah. The BSAFs are greater than one in the pheasant coucal for the lower chlorinated PCDDs and the PCBs, but less than one for most PCDF congeners.

Figure 3.4 shows the BSAFs for each of the 29 dioxin-like congeners in different species of raptors collected in South Australia. The BSAFs vary among species by about 1 order of magnitude. The highest bioaccumulation generally occurs in the sparrowhawks, while the

lowest occurs in the goshawk. The differences in the BSAF among individual raptors of the same trophic level could result from a number of factors including individual and species differences in behaviour, habitat, size of the home range, levels of dietary exposure, and differences in age, gender and other physiological characteristics. The small number of samples does not allow firm conclusions to be drawn about the effect of species on BSAF.

The pattern of variation in BSAFs between individual congeners generally holds for all bird species (Figure 3.3 and 3.4). The more toxic PCDD and PCDF congeners have higher BSAFs, indicating greater biomagnification potential, than the hepta and octa-chlorinated congeners. PCDD congeners generally having the highest BSAFs are 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD and 1,2,3,6,7,8-HxCDD. The PCDF congener 2,3,4,7,8-PeCDF consistently had the highest BSAF. In addition, BSAFs for PCDDs appear to be highest in urban birds than in birds collected from agricultural or remote areas. While BSAFs for PCDFs and PCBs are higher in birds from agricultural and remote sites, at least in South Australian birds.

The PCBs are biomagnified in birds to an even greater extent that dioxins and furans. The BSAFs for PCBs are in general between 1 to 3 orders of magnitude higher in birds of prey than in soil. Bioaccumulation and persistence of PCBs in birds is related to their chlorine substitution pattern, with PCB congeners containing adjacent chlorine atoms in the *meta-para* positions (i.e. positions 3.5) on either phenyl ring being highly persistent (Drouillard et al., 2001).

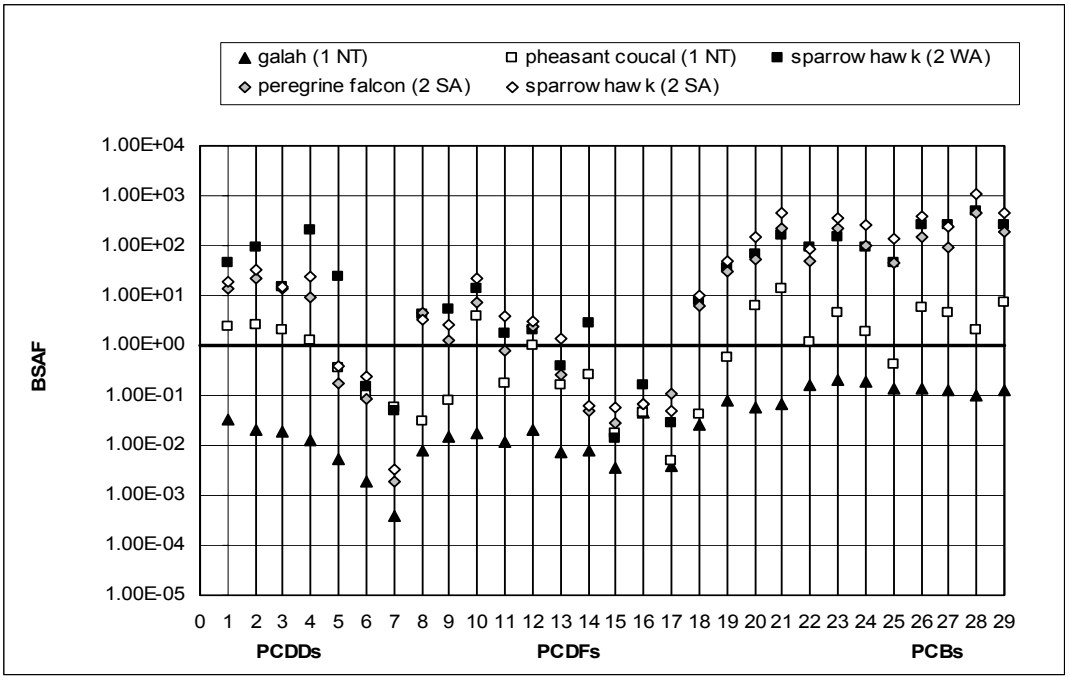



**Figure 3.3 BSAFs for birds from different trophic levels and regions compared to soils within those regions**

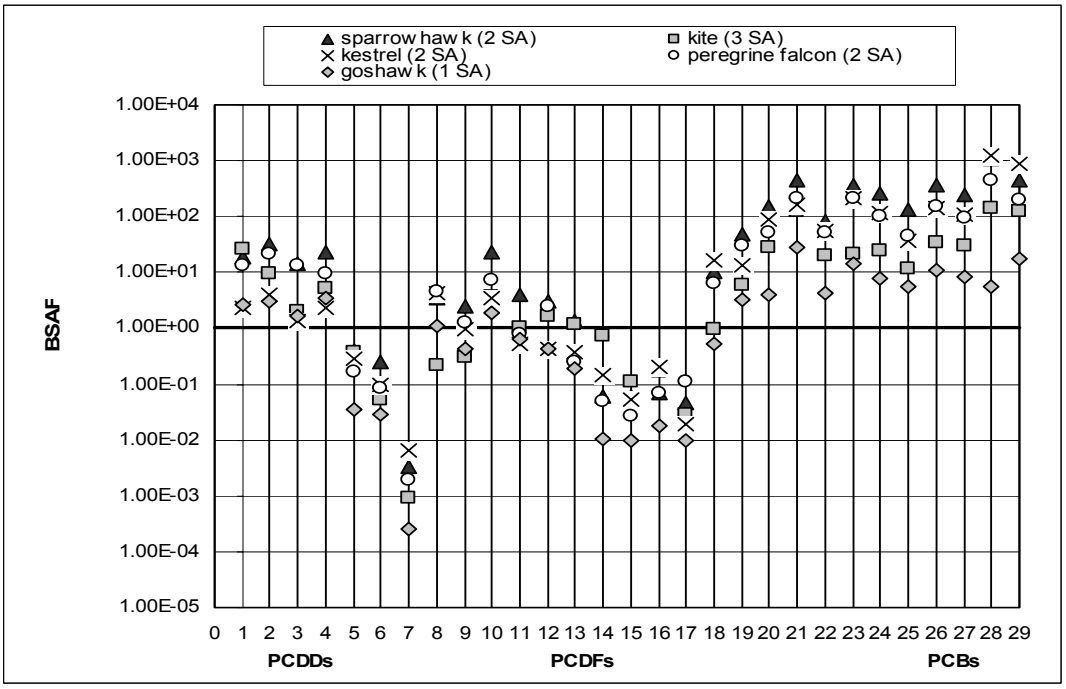



**Figure 3.4 BSAFs of PCDDs (1-7), PCDFs (8-17) and PCBs (18-29) for different species of raptors from South Australia compared to South Australian soils**

PCB 77 consistently had the lowest BSAF in birds. Congeners showing consistently high BSAFs in urban birds were PCB 169, PCB 114, PCB 156, PCB 167, and PCB 189. Congeners showing high BSAFs in birds from agricultural and remote areas also included PCB 126, PCB 105, PCB 118, and PCB 167. These generalisations, are however, based on small samples sizes, and hence, should not be considered conclusive.

## 3.5 Aquatic exposure

Water living organisms can accumulate pollutants either directly from the surrounding environment, or indirectly from contaminated food. For dioxins, uptake in food is the most important source of exposure, with direct uptake from water via gills and skin expected to be negligible owing to their very low aqueous solubility (Gobas, 1993).

It has been shown that sorption of chemicals to suspended and dissolved organic matter reduces their bioavailability to pelagic organisms, such as fish. However, sediment-sorbed chemicals can be ingested and accumulate in benthic organisms, from where they are transferred through the food chain to pelagic species at higher trophic levels (Loonen et al., 1997). Microorganisms, such as bacteria, viruses, flagellates ciliates, phytoplankton, and microzooplankton, as well as the larger benthic organisms, are the important first links for pollutant transfer in pelagic aquatic food webs. For example, small fish and crustaceans eat the micro- and benthic organisms, and then larger fish and cephalopods eat the smaller fish. Piscivorous mammals, such as dolphins, certain whales and seals in turn consume the larger predator fish, squid, and octopus. Other predators, such as sharks and killer whales, may

subsequently eat the piscivorous mammals. Fifth and sixth order consumers are not unusual in aquatic food chains.

### 3.5.1 Sediment

Dioxin levels were determined in 75 sediment samples from 58 locations within Australian catchments with site categories of freshwater, estuary, and marine aquatic environments (Table 3.8).

**Table 3.8 Total PCDD, PCDF and PCBs, expressed as TEQs, in Australian sediments**

| | | Geographic region | | | |
|---|---|---|---|---|---|
| **Landuse** | **pg/g dw** | **Northern** | **Southeastern** | **Southwestern** | **All** |
| Marine | Range | 0.029 – 0.037 | 0.03 – 4.2 | 0.11 – 0.13 | 0.029 – 4.2 |
| (n=12) | Median | 0.033 | 0.19 | 0.12 | 0.12 |
| | Mean | 0.033 | 0.97 | 0.12 | 0.67 |
| Freshwater | Range | 0.061 – 0.37 | 0.04 – 1.3 | 0.14 – 3.1 | 0.042 – 3.1 |
| (n=33) | Median | 0.14 | 0.23 | 0.2 | 0.2 |
| | Mean | 0.18 | 0.45 | 1.02 | 0.53 |
| Estuarine | Range | 0.14 – 6.8 | 0.16 – 520 | 0.066 – 5.5 | 0.066 – 520 |
| (n=30) | Median | 0.77 | 4.9 | 3.6 | 2.3 |
| | Mean | 1.63 | 49 (23)* | 3.06 | 32 |

*Mean excludes Parramatta River

Dioxins were found in Australian sediment in concentrations ranging from 0.002 pg TEQ/g dw to 520 pg TEQ/g dw (½ LOD, mammalian TEFs). Highest concentrations were found in sediments from the urban and industrial regions of the Parramatta River estuary (100 and 520 pg TEQ/g dw) and the western section of Port Jackson, NSW (78 and 100 pg TEQ/g dw). Other estuarine waters with somewhat elevated TEQ concentrations include Botany Bay (35 pg TEQ/g dw), as well as the estuaries near Brisbane, Melbourne, Hobart, Perth and Wollongong (5.5 to 17 pg TEQ/g dw). The median concentrations for all sediments were 0.2, 2.3 and 0.12 pg TEQ/g dw for freshwater, estuarine and marine locations, respectively (Müller et al., 2003).

The sample from the Parramatta River is not strictly representative of urban estuarine background levels. The Parramatta River is in the vicinity of Homebush Bay, which is a recognized hot spot for dioxin contamination resulting from chemical manufacturing in the area from 1928 to 1986. The site at Homebush Bay is about to be remediated. The current bay-wide average levels of dioxin in the Homebush Bay surface sediments has been estimated at approximately 3000 pg TEQ/g. As a consequence, the surface sediments in the Parramatta River are also contaminated with dioxins, which when expressed as total TEQ, has been estimated to be 490 pg/g dry weight (Cleland, 2003).

As with soils, the congener profiles in sediment were, for most samples, dominated by the higher chlorinated PCDDs/Fs, particularly OCDD. However, in some urban estuaries, PCBs contributed up to 50% to TEQ. The profiles for PCBs were dominated by PCB congeners 118, PCB 105, PCB 156 and PCB 77.

### 3.5.2 Bivalves and fish

The NDP fauna survey provided tissue concentrations for 18 bivalves, 23 fish, and 13 marine mammals. Marine mammal samples were obtained from various locations and comprised high trophic level species including (beached) whales, an Australian sea lion and two species of dolphins. A dugong, which is an aquatic herbivore, was also included to provide a contrast of the trophic levels. Commercially available fish and bivalve samples were collected from as near as possible to the sediment sampling locations.

**Bivalves**

Bivalves are common shallow-water molluscs, and include scallops, oysters, and clams. Bivalves are mostly benthic (bottom dwelling) and marine, although some live in fresh water. Dioxin levels were measured in mussels and oysters from estuarine waters (n=11), the marine environment (n=6), and included one from a freshwater ecosystem. Results are shown in Table 3.9.

Oysters and mussels are sedentary filter feeders that depend on currents created by cilia in their gills to provide them with food from the surrounding water. While dioxins are not toxic to bivalves, they provide a food source for other aquatic organisms able to break open the shells, for example, shore birds such as the oyster catchers, which feed on intertidal mussels and oysters. As such, the TEQs are calculated for comparison of dietary exposure levels assuming TEFs for birds and fish.

**Table 3.9 Total PCDD, PCDF and PCBs, expressed as TEQs, in bivalves from freshwater (F), estuarine (E) and marine (M) environments**

| | | pg TEQ/g | | |
|---|---|---|---|---|
| **Species** | **Sampling location** | **Wet weight** | **Lipid weight** | **% lipid** |
| Mussels | Port Jackson West, Sydney (E) | 1.2 | 50 | 2.4 |
| Oysters | Coffin Bay (M) | 0.9 | 37.5 | 2.4 |
| Oysters | Lower Yarra R, Melbourne (E) | 0.67 | 35 | 1.9 |
| Oysters | Eastern Moreton Bay (M) | 0.41 | 34 | 1.2 |
| Oysters | Port of Darwin (E) | 0.38 | 12.3 | 3.1 |
| Oysters | Port of Brisbane, Brisbane (E) | 0.25 | 16.7 | 1.5 |
| Oysters | Lower Tamar R, Launceston (E) | 0.22 | 11.6 | 1.9 |
| Oysters | Redland Bay (E) | 0.20 | 20 | 1.0 |
| Mussels | Hobsons Bay, Melbourne (E) | 0.19 | 20.6 | 0.92 |
| Mussels | Port Phillip Bay East Melbourne (M) | 0.13 | 17.6 | 0.74 |
| Oysters | Lower Johnstone R, Innisfail (E) | 0.13 | 9.3 | 1.4 |
| Mussels | Lower Derwent R, Hobart (E) | 0.086 | 9.5 | 0.90 |
| Mussels | Gippsland Lakes (E) | 0.07 | 1.6 | 4.3 |
| Oysters | Heron Island (M) | 0.04 | 4.4 | 0.90 |
| Mussels | Upper Brisbane R (F) | 0.03 | 1.7 | 1.8 |
| Mussels | Kwinana Beach, Perth (M) | 0.02 | 2 | 1.0 |
| Oysters | Coffin Bay (M) | 0.02 | 0.54 | 3.7 |
| Mussels | Lower Torrens R, Adelaide (E) | 0.008 | 1.6 | 0.50 |

The concentrations of dioxins in bivalve samples from different regions ranged from 0.008 pg TEQ/g wet weight (ww) to about 1.17 pg TEQ/g ww, when expressed using the fish TEFs and including the full LOD. The mean was 0.28 pg TEQ/g ww and the median was 0.08 pg TEQ/g ww. The highest concentrations occurred in samples from Port Jackson, Sydney. The concentrations in bivalves using avian TEFs and the full LOD ranged between 0.052 – 38.3 pg TEQ/g ww, with a median of 0.686 and a mean of 4.7 pg TEQ/g ww. The large difference in TEQ values calculated using avian TEFs results mainly from the higher toxicity of PCB 77 to birds. PCB 77 has a TEF of 0.0001 for fish, and 0.05 for birds. Other congeners with significantly higher toxicity to birds than fish include PCB 81, PCB 126, and 2,3,7,8-TCDF.

**Fish**

Tissue concentrations are available for 12 different fish species. These comprise pelagic (open water) and estuarine species, and freshwater fish. The predominant route of dioxin exposure to fish in the wild is expected to be through the diet, with direct waterborne exposure through the gills likely to be a minor route of uptake owing to the insolubility of dioxins in water. The fish species sampled live in a range of habitats and consume a range of foods. For example, barrumundi live in creeks, rivers and estuaries in clear or turbid water, and eat mainly fish, crustaceans and aquatic insects. Flathead (no species provided) live in estuarine and coastal water but are generally bottom dwellers. King George Whiting inhabit the shallow waters of the inner continental shelf and inlets, feeding on bethic amphipods, crustaceans, polycheate worms and molluscs. The Australian salmon (unrelated to northern hemisphere salmon) are pelagic fish, although it is thought that young fish spend the first two years of their life in estuaries and inlets. These fish eat other fish (e.g. pilchards, sardines, mullet, garfish), as well as krill, squid, whitebait, shellfish and worms.

The concentrations of dioxins in fish are provided in Table 3.10. Dioxin levels in fish ranged from 0.009 pg TEQ/g wet weight to about 0.49 pg TEQ/g ww, using fish specific TEFs and the full LOD. The mean was 0.09 and the median was 0.045 pg TEQ/g ww. The TEQs in fish reported on a lipid weight basis ranged between 1.4 and 60 pg/g lipid. The concentrations in fish using avian TEFs and the full LOD ranged between 0.0095 and 0.49 pg TEQ/g ww, with a median of 0.045 and a mean of 0.09 pg TEQ/g ww.

No information was provided in the NDP survey report on the site category for fish sampling locations. The highest concentration found in fish on a wet weight basis was found in a whiting sample obtained from the Sydney/Port Jackson area. This site also had the highest dioxins levels in sediments. The highest concentration in fish on a lipid weight basis was found in a barramundi from the Gulf of Carpentaria. This individual had a very low lipid content. High concentrations were also found in Golden perch from Coopers Creek. The difference in TEQ concentrations in fish is likely to be related to feeding behavior and site differences in contamination of food. Russell et al., (1999b) found benthic feeding fish were consistently more contaminated than pelagic piscivorous fish, in aquatic food chains probably because benthic feeding fish ingest large quantities of sediment with their food.

**Table 3.10 Total PCDD, PCDF and PCBs, expressed as TEQs, in fish from freshwater, estuarine, and marine locations**

| | | pg TEQ/g | | |
|---|---|---|---|---|
| **Species** | **Location** | **Wet weight** | **Lipid weight** | **% lipid** |
| King George Whiting, *Sillaginodes punctata* | Port Jackson | 0.49 | 10.7 | 4.6 |
| Golden Perch, *Macquaria ambigue* | Coopers Creek | 0.36 | 18 | 2.0 |
| Barramundi, *Lates calcarifer* | Gulf of Carpentaria | 0.24 | 60 | 0.40 |
| River Cobbler, *Cnidoglanis macrocephalus* | Albany Region | 0.10 | 10 | 1.0 |
| King George Whiting, *Sillaginodes punctata* | Melbourne Region | 0.096 | 5.6 | 1.7 |
| Flathead, *Platycephalus spp.* | Gippsland Lakes | 0.089 | 1.4 | 6.4 |
| Australian Salmon, *Arripis truttaceus* | Hobart - Storm Bay | 0.08 | 1.3 | 6.2 |
| Barramundi, *Lates calcarifer* | Darwin Region | 0.078 | 6.5 | 1.2 |
| Bream, *Dentex spp.* | Moreton Bay | 0.061 | 6.1 | 1.0 |
| Sand Whiting, *Sillago cilitia* | Moreton Bay | 0.058 | 9.5 | 0.61 |
| Barramundi, *Lates calcarifer* | Cairns Region | 0.054 | 5.4 | 1.0 |
| Flathead, *Platycephalus spp.* | Melbourne Region | 0.043 | 4.7 | 0.91 |
| Flathead, *Platycephalus spp*. | Moreton Bay | 0.04 | 4.9 | 0.82 |
| Green Backed Flounder, *Rhombosolea tapirina* | Melbourne Region | 0.04 | 2.1 | 1.9 |
| Flathead, *Platycephalus spp.* | Triabunna Region | 0.04 | 1.2 | 3.4 |
| Flathead, *Platycephalus spp*. | Albany Region | 0.04 | 4 | 1.0 |
| Golden Perch, *Macquaria ambigue* | Murray River | 0.036 | 2.8 | 1.3 |
| Short-finned Eel, *Anguilla australis* | Latrobe River | 0.033 | 2.8 | 1.2 |
| Flathead, *Platycephalus spp.* | Port Jackson | 0.03 | 1.5 | 2.0 |
| Barramundi, *Lates calcarifer* | Roebuck Bay | 0.025 | 1.9 | 1.3 |
| Garfish, *Hyporhamphus australis* | Adelaide Region | 0.017 | 0.68 | 2.5 |
| Flathead, *Platycephalus spp.* | Adelaide Region | 0.01 | 0.7 | 1.4 |
| Bream, *Dentex spp.* | Port Jackson | 0.009 | 0.69 | 1.3 |

### 3.5.3 Marine mammals

Table 3.11 provides the tissue concentrations of dioxins found in marine mammals during the NDP survey. Total TEQs (lipid weight basis, full LOD, mammalian TEFs) ranged between 1.85 pg/g lipid in the dugong to 585 pg/g lipid in the bottlenose dolphin blubber from Port Adelaide. The humpback dolphin (*Sousa chinensis*) from Darwin Harbour had the second highest value of 190 pg TEQ/g lipid. The TEQs in other piscivorous mammals were remarkably similar. The mean TEQ for all marine mammals was 82.3 and the median 28.8 pg TEQ/g lipid.

Levels of PCDD/F were below the detection level for most congeners, except for the higher chlorinated compounds. OCDD was the dominant PCDD/F congener. Other congeners occurring at significant but lower levels included 1,2,3,6,7,8-HxCDD and 1,2,3,4,6,7,8-HxCDD. The dolphin from the Northern Territory was the exception in that most other PCDD congeners and 2,3,7,8-PCDF were also present at significant levels. PCBs contributed the highest TEQ load in all marine mammals, except the dugong. The dominant PCB congeners were PCB 118 (53 to 74 % total PCBs), PCB 105 (11 to 22%), and PCB 156 (<14%), and PCB 167 (<85%).

Other studies have reported the contribution of coplanar PCBs to TEQs, relative to dioxins and furans, is significantly higher in marine mammals than in terrestrial mammals (Tanabe et al., 1994).

The lower levels of dioxins in the dugong reflect its low trophic level. Dugongs are aquatic herbivores that graze exclusively on sea grass, and are only likely to become exposed by consuming contaminated sediment along with the sea grass. Other studies with marine mammals also show that body burdens of organic chemicals are lower in herbivores than fish-eating species such as dolphins, and pilot and belugas whales. The TEQs found in the dugong in the NDP survey are, however, lower than those reported by Gaus et al., (2001b) for dugongs from the Queensland coast, where TEQs from PCDD/Fs were in the range 1.5 to 135 pg/g (average 34 pg/g lipid, n = 16).

The large differences in tissue concentrations found in marine mammals of the same trophic level probably reflect differences in the levels of contamination in food between habitat types and geographic areas. For example, the higher levels of dioxins found in dolphins may reflect the influence of local contamination. The dolphin's habitat incorporates coastal and estuarine environments as well as the deeper marine environment. Dolphins living in near coastal environments may be more vulnerable to exposure to dioxins from urban and industrial runoff. Both dolphins were collected near urban ports (Darwin Harbour and Port Adelaide), whereas the Australian sea lion was collected from Kangaroo Island, a habitat with considerably less industrial influence (Correll et al., 2003). Whales prefer the deeper offshore marine environment, where they are less likely to encounter contaminated fish. Whales were collected from the relatively pristine coastal waters of Tasmania.

**Table 3.11 TEQs in aquatic mammals**

| | | pg TEQ/g lipid | | |
|---|---|---|---|---|
| **Species** | **Sampling location** | **PCDD/F** | **PCB** | **Total** |
| Bottlenose dolphin blubber, *Tursiops aduncus* | Port Adelaide, SA | 4.6 | 580 | 585 |
| Humpback dolphin, *Sousa chinensis* | Darwin, NT | 15 | 175 | 190 |
| Sperm Whale, *Physeter catodon* | Waterhouse, TAS | 6.1 | 36 | 42 |
| Sperm Whale, *Physeter catodon* | Waterhouse, TAS | 5.5 | 34 | 39 |
| Sperm whale, *Physeter sp* | West Coast, TAS | 6.6 | 30 | 37 |
| Sperm Whale, *Physeter catodon* | Waterhouse, TAS | 4.7 | 26 | 31 |
| Sperm Whale, *Physeter catodon* | Waterhouse, TAS | 4.7 | 24 | 29 |
| Beaked whale, *Physeter sp* | Cloudy Bay, TAS | 6.2 | 21 | 27 |
| Sperm Whale, *Physeter catodon* | Waterhouse, TAS | 1.1 | 23 | 24 |
| Australian sea lion, *Neophoca cinerea* | Seal Bay, SA | 1.7 | 22 | 23 |
| Long fin pilot whale, *Physeter sp* | Sisters Beach, TAS | 3.9 | 17 | 21 |
| Sperm Whale, *Physeter catodon* | Waterhouse, TAS | 7.2 | 13 | 20 |
| Dugong, *Dugong dugong* | Darwin, NT | 1.5 | 0.34 | 2 |

The TEQs in dolphin blubber are higher than those reported by Gaus et al., (2001b) for dolphins (n = 5) from the Queensland coast, where TEQs ranged from 1.1 to 4.3 pg/g, with an average of 2 pg/g. However, this is most likely because PCBs were not measured in the Gaus et al., study and therefore only PCDD/Fs contributed to the TEQs.

The age and gender of marine mammals can influence the amount of residues that accumulate, all else being equal. However, data on the age and gender of samples was not available in the NDP survey. Age influence the amounts of residue accumulated because marine mammals are long-lived and as such integrate exposures over relatively long time frames. For males, there is typically an increase in accumulation of persistent organic pollutants in the body with age, while for breeding females there is a decrease after maturity due to losses during lactation (Tanabe et al., 1994).

## 3.6 Biota-sediment accumulation factors for aquatic fauna

### 3.6.1 BSAFs for bivalves

Figure 3.5 shows the BSAFs for each dioxin-like congener calculated for selected bivalve samples from various locations in Australia, and the sample average (n = 17). The ratios were calculated by dividing the tissue concentrations in bivalves by the concentrations in surface sediment sample collected in the vicinity of the organism.

On average, BSAFs are generally less than one for PCDDs and PCDFs, but are greater than one for PCBs. BSAFs tend to initially increase with increasing chlorination (penta and hexa) and then decrease again with the highest chlorinated congeners (hepta and octa). The highest BSAFs are seen in mussels from Gippsland Lakes, with PCB 77, PCB 105, PCB 118, and PCB 167 having the highest ratios (ranging from 7 to 25). The BSAFs for bivalves collected in Port Jackson are lower than expected given that the sediment had the highest concentration of all sediments. However, a high BSAF does not necessarily equate to a high body burden. BSAFs are based on simple ratios (relative concentrations). A soil sample with a high exposure concentration could result in a lower BSAF relative to a sample with a lower exposure concentration, if the lower exposed organism accumulates relatively more of the available contaminant. At high exposures, not all of the amount of chemical in the sediment is necessarily bioavailable and actually absorbed by the organism, although more may be taken up in real terms than in the low exposure sample with higher BSAFs.

Other studies have reported lower than expected BSAFs for PCDD/Fs in mussels for the concentrations available in sediment (Hayton et al., 1990; Segestro et al., 1995). These authors attributed this to the ability of bivalves to close up and cease feeding during periods of poor water quality and sediment resuspension, and thereby prevent uptake of dioxins. Other factors that control the BSAF include site-specific factors such as the age of the contamination. Bioavailability is often found to be lower in field situations, where longer sediment incubation times occur. For example, Loonen et al. (1997) reported steady-state BSAFs of 1.6 for TCDD and 0.07 for OCDD in oligochaetes worm (*Lumbriculus variegatus*) after 28 days exposure in the laboratory. Exposure to older sediments, which had been in contact with PCDDs for two years, resulted in much lower BSAFs after 28 days.

BSAFs (for total PCBs rather than individual congeners) of between 2.1 and 10.4 have been reported (based on lipid and organic carbon normalised concentrations) in the Great Lakes system (US EPA, 2000c appendix). Ferraro et al., (1991) reported BSAF for marine clams from 0.22 to 0.68 for PCB 105, from 0.54 to 4.74 for PCB 118, and from 0.16 to 0.67 for PCB 156.

### 3.6.2 BSAFs for fish

Figure 3.6 shows the BSAFs of each of the 29 dioxin-like congeners in selected fish. The fish samples were obtained from commercial fishermen, and were caught in close proximity to the sediment sampling locations (Müller et al., 2002b). The concentration ratios for fish, were therefore, calculated by dividing the tissue concentrations in fish by average concentrations in surface sediment in the vicinity of the organism. The number of sediment samples available for each area was small, ranging between one to three samples.

BSAFs were generally higher for bivalves than for fish. BSAFs for crustaceans and molluscs tend to be higher than for fish because of their close proximity to sediment-associated dioxins (Comber et al., 2003). According to Burkhard et al. (2000) equilibrium partitioning theory suggests that invertebrates in intimate contact with the soils should have BSAFs in the range of one to four for chemicals of all $K_{ow}$s in the absence of metabolism.

For organisms, such as fish and crustaceans, not in contact with the sediment, the BSAF will depend on a number of site-specific parameters, including the relative contributions of pelagic and benthic organisms in their diet, kinetic limitations for chemical transfer from sediment to water, such that surface-sediment concentrations have not reached steady-state with water, and biological processes such as biotransformation or biomagnification (US EPA, 1993a; Burkhard et al., 2000).

BSAFs for fish are less than one for all congeners, with a notable exception being the BSAF for PCBs in the Australian salmon from the Derwent River. In general BSAFs decrease for the highest chlorinated congeners. Bioaccumulation kinetics in fish is influenced by the lipid content, size, and age. Dioxins are accumulated in animals with large amounts of fat. The salmon has a relatively high lipid content (6.2%), as does the flathead from Gippsland Lakes (6.4%), which would account for the higher accumulation of PCBs in these fish relative to sediments. The King George Whiting from Port Jackson has the third highest lipid content of 4.6%. All other fish have a lipid content below 3%. The fish sample from Port Jackson has the highest TEQ (0.49 pg/g wwt), as do the sediments samples from Port Jackson. However, the levels in the fish are much lower relative to levels in the sediment, making the BSAF for this sample lower than for other samples with a lower TEQ.

Most fish BSAFs reported in the literature for TCDD, are less than 1.0. For example, the lake wide average BSAFs reported for TCDD in Lake Ontario fish species were; lake trout - 0.07, brown trout - 0.03, yellow perch - 0.03, white perch - 0.20, smallmouth bass - 0.05, smelt - 0.06 and slimy sculpin - 0.12 (US EPA, 1993a).

There was a tendency for deeper water fish (lake trout, sculpin, smelt) to have greater lipid-normalised TCDD concentrations than near-shore, shallower water species, including brown trout (*Salmo trutta*), yellow perch (*Perca flavescens*) and smallmouth bass (*Micropterus salmoides*). White perch (*Morone americana*), an introduced species were found to have the greatest concentration of TCDD, at least in part due to their age.

Schell et al., (1993) report BSAFs for 2,3,7,8-TCDD of 0.088-0.1, 0.18-0.26 and 0.04-0.07 for largemouth bass (*Micropterus salmoides*), bowfin (*Amia calva*) and brown bullhead

catfish (*Ictalurusbebulosus*) respectively. These values are for TCDD in the liver of a composite sample of a field population. Endicott and Cook (1994) report a BSAF of 0.21 for TCDD derived from a composite sample of lake trout (*Salvelinus namaycush*) collected from Lake Ontario which were then exposed to historically contaminated sediments.

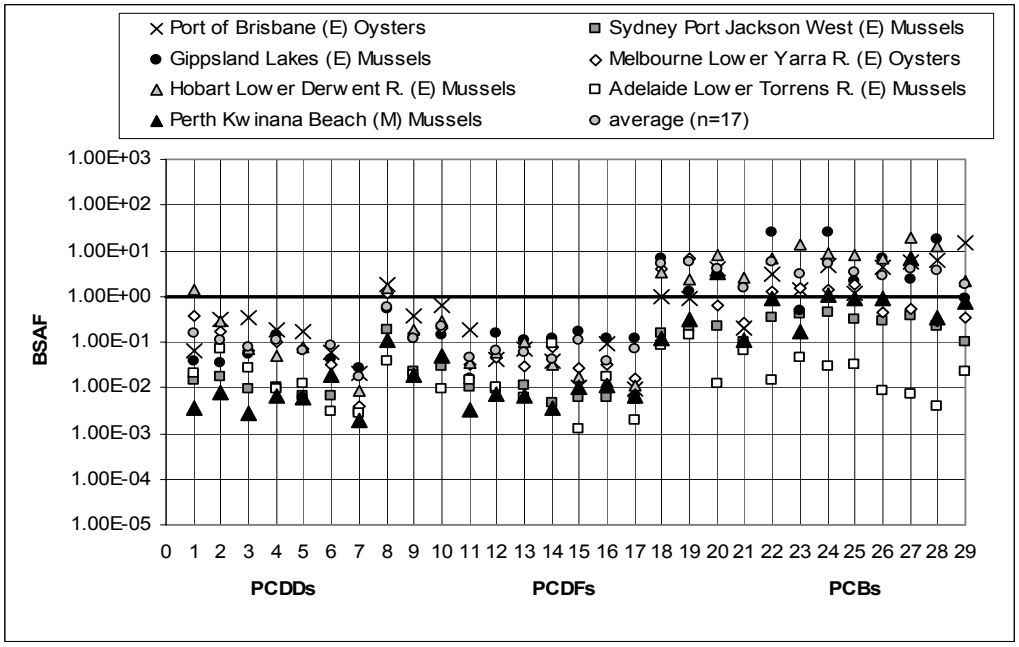
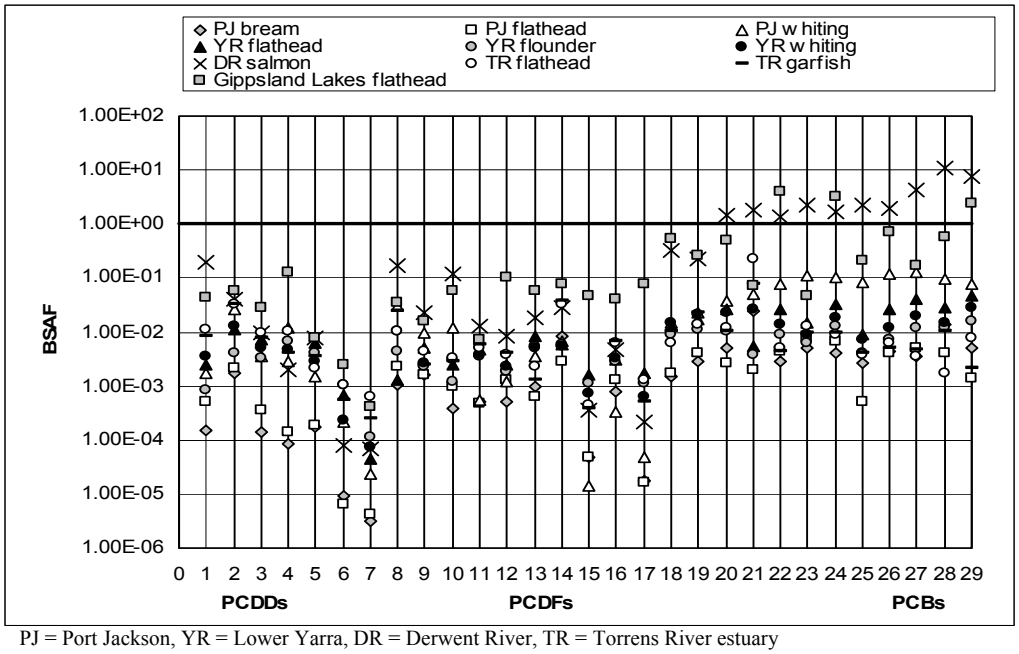



**Figure 3.5 BSAFs of PCDDs (1-7), PCDFs (8-17) and PCBs (18-29) for bivalves**




PJ = Port Jackson, YR = Lower Yarra, DR = Derwent River, TR = Torrens River estuary

**Figure 3.6 BSAFs of PCDDs (1-7), PCDFs (8-17) and PCBs (18-29) for fish.**

### 3.6.3 BSAFs for marine mammals

Figure 3.7 shows the BSAFs of each of the 29 dioxin-like congeners in marine mammals. The ratios were calculated by dividing the tissue concentrations in marine mammals by average concentrations in marine sediment. As with predatory birds, marine mammals are expected to range over a wide area in search of food and as such would encounter a wide range of exposure conditions by consuming a variety of fish with a range of accumulations.

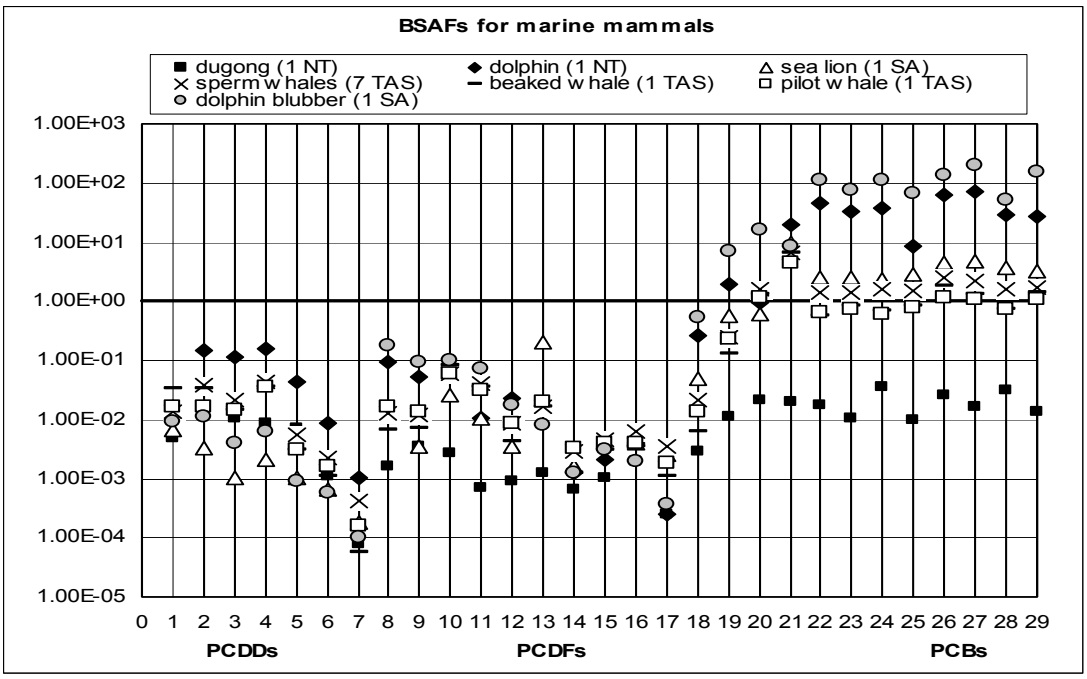



**Figure 3.7 BSAFs of PCDDs (1-7), PCDFs (8-17) and PCBs (18-29) for marine mammals (n = 13) compared to marine sediments (n=12)**

For dioxins and furans, all BSAFs are less than one, indicating no biomagnification is occurring relative to sediment. For PCBs, the BSAFs are greater than one in the dolphins, sea lions, and sperm whales. The lowest BSAF is found in the dugong and the highest BSAF is found in the dolphin samples. These differences appear to be related to trophic level.

## 3.7 Comparison with other studies

### 3.7.1 Bioaccumulation

Numerous bioaccumulation studies have been published for dioxins. A large proportion of these have been concerned with measuring bioconcentration of TCDD in fish from water under controlled laboratory conditions. Field studies have also been undertaken to examine bioaccumulation of dioxins in aquatic environments. The relevant field studies have generally determined accumulation of dioxins in fish and other aquatic organisms directly from sediment or by consuming their prey. In addition to these studies, models to predict

bioaccumulation of dioxins through different trophic levels of the aquatic food chain have been developed based on their chemical properties (i.e. $K_{ow}$). However, only a few of these models have undergone field validation. Very few studies are available focusing on terrestrial food-chain bioaccumulation of dioxins. The lack of information on terrestrial bioaccumulation is cited as an important information gap in contaminant exposure and risk assessment of dioxins (Kelly and Gobas, 2003).

The literature on bioaccumulation of dioxins clearly shows a lack of standardisation among studies, making comparisons between studies very difficult. The main inconsistencies are associated with the way bioaccumulation is expressed. For example, in more recent studies, congener concentrations in organisms are reported on a lipid weight basis, while in older studies they are normally reported on a wet weight basis. Soil and sediment concentrations are reported on either a dry weight or organic carbon-normalised basis. Some studies report dioxin concentrations in the liver or other specific organ, rather than in the whole organism or it's lipid. Many studies provide concentrations only for TCDD or for total PCBs, which may include non-dioxin like PCBs. Where concentrations are reported in terms of TEQs, these are often based on different TEF systems. Bioaccumulation ratios have been calculated from any combination of these measures.

This lack of standardisation among studies exists in the main because of the varying ages of the literature studies, and the continuing development of analytical technology. While older studies used the best technology available at the time they were conducted, major advances made in analytical technology in the last decade, has greatly improved the sensitivity of the analytical methods for measuring dioxin-like congeners. The recent development of a scientific consensus TEF system has also meant older systems have become outdated.

The available field-based aquatic bioaccumulation studies generally show a progressive increase in tissue PCDD/Fs and PCBs from low to high trophic level consumers. For example, Harihiko et al., (2003) measured sediment and tissue concentrations of selected PCBs in a tidal flat ecosystems in western Japan comprising sediment, clams, crabs, mudskippers, flatfish, coastal water fish, squid and finless porpoises (Table 3.12). Their results indicated a trend of increasing PCB concentrations with trophic level, with the highest levels found in the finless porpoises. Lower accumulation is seen in coastal pelagic fish compared to organisms living in the mudflats. The dominant PCB congeners were the co-planar PCBs, PCB 118 and PCB 105. Interestingly, these congeners were also dominant in Australian fauna.

Bioaccumulation of TCDD in the Great Lakes (North America) food chain has been the focus of numerous studies. These studies have shown that biomagnification of TCDD in Lake Ontario food chains is significant between fish and fish-eating birds but not between fish and their food, or fish and sediment. BSAFs for TCDD in fish are approximately 20-fold less than for PCBs. Benthic invertebrates have larger BSAFs for TCDD than fish (US EPA, 1993a).

Jones *et al* (1993) measured bioaccumulation of TCDD-EQ and PCBs in different trophic levels in the Great Lakes ecosystem. Biomagnification factors and biota-sediment

accumulation factors derived from this study are provided in Table 3.13. Their results showed concentrations of PCBs and TCDD-EQ increased in each trophic level. The degree of accumulation in organisms was more closely linked to the diet than to the species. Fish and bird species with similar feeding habits had similar levels of TCDD-EQ (e.g. red-breasted merganser and double-crested cormorant), while closely related species with different feeding habits (e.g. mallard and red-breasted merganser) had very different TCDD-EQ concentrations.

**Table 3.12 PCBs (ng/g lipid) and BSAFs in tidal flat and coastal organisms of different trophic levels in Japan**

| Media | PCB 105 | BSAF | PCB 118 | BSAF | PCB 156 | BSAF |
|---|---|---|---|---|---|---|
| Sediment ng/g OC | 4.76 | - | 24 | - | <2.4 | |
| Clam | <8.47 | 1.8 | 22 | 0.9 | <8.5 | 3.5 |
| Crab | 53 | 11 | 103 | 4.3 | 16.7 | 7.0 |
| Mudskipper (herbivore) | 61 | 12.8 | 77 | 3.2 | 11.3 | 4.7 |
| Mudskipper (omnivore) | 74 | 15.5 | 148 | 6.2 | 17.8 | 7.4 |
| Mudskipper (omnivore) | 44 | 9.2 | 114 | 4.8 | 15.8 | 6.6 |
| Coastal water fish | 9.6 | 2.0 | 26 | 1.1 | 1.8 | 0.75 |
| Squid | 5.95 | 1.2 | 7.1 | 0.3 | 5.9 | 2.5 |
| Porpoise | 310 | 65 | 1126 | 47 | 62 | 26 |

**Table 3.13 BMFs and BSAFs at different trophic levels in the Great Lakes ecosystem**

| Trophic level | Mean PCB (µg/g) | BMF | BSAF | Mean TCDD-EQ (pg/g) | BMF | BSAF |
|---|---|---|---|---|---|---|
| Sediment (0) | 1.6 | - | - | 10.2 | - | - |
| Fish (3) | 0.2 | 0.1 | 0.1 | 10.0 | 1.0 | 1.0 |
| Fish and juvenile birds (4) | 1.1 | 5.5 | 0.7 | 77.3 | 7.7 | 7.6 |
| Adult fish-eating birds (5) | 22.7 | 20.6 | 14.2 | 314.3 | 4.1 | 30.9 |

BMF = concentrations in trophic level (n)/concentration in trophic level (n-1), BSAF = concentrations in trophic level (n)/concentration in sediment. Trophic level assignment: sediment = 0, algae = 1, plankton = 2, planktivores = 3, piscivorous fish = 4, piscivorous birds = 5. Trophic level 3 comprised alewife (*Alosa pseudoharengus*), smelt (*Osmerus mordax*), whitefish (*Coregonus cluoeaformis*). Trophic level 4 comprised herring gull (*Larus argentatus*), cormorant (*Phalacrocorax auritus*), ring-bill gull (*Larus delawarensis*), mallard (*Anas platyrhynchos*), and Lake trout and Northern pike. Trophic level 5 comprised herring gull, cormorant, ringbill gull and merganser (*Mergus serator*).

In arctic aquatic food chains, BMFs for PCBs of 3.7 to 8.8 were determined for trophic transfer from fish to seal, BMFs of 7.4 to 13.9 were determined for transfer from seal to bear, and BMFs of 49.2 were determined for transfer from fish to bear (Muir et al., 1988). In another study, Traas et al., (2001) modeled bioaccumulation of PCBs in the body lipid of otters using BSAFs for different fish species, and BMFs for transfer from fish to otter. Bioaccumulation of PCBs resulted in median TEQs in otter of about an order of magnitude higher than in the average diet. The median TEQ in the diet was again an order of magnitude higher than in sediment. PCB 126 and PCB 169 were selectively retained by otters in comparison to the mono-ortho-PCBs. Bioaccumulation in males was higher than in females. This was mainly attributed to loss of PCBs during lactation. A positive

correlation was found between age and PCB concentrations. For females accumulation with age does not usually occur.

### 3.7.2 Maternal transfer studies

Bird embryos are exposed to dioxins through maternal transfer at the time of egg formation. Dioxins present in the mother are transferred along with fat to the yolk of the egg. Most toxicity reference values (TRV) for birds are expressed as contaminant concentrations in eggs rather than concentrations in adult birds, reflecting the greater sensitivity of bird embryos than adults to the toxic effects of dioxins.

A limited number of studies have compared the proportion of the maternal dioxin burden transferred from the maternal tissue of birds and their eggs. A study by Russell et al. (1999) indicates that for oviparous (egg producing) organisms, lipid adjusted chemical concentration in eggs and maternal tissue are fairly close, and typically are within a factor of two for lipid soluble organic chemicals (for fish this value is typically equal to 1.0, while for birds the value is nearer to 0.6).

For example, Braune and Norstrum (1989) measured egg and whole-body concentrations PCDD/Fs and PCBs in herring gull (*Laurus argentatus*) collected from the organochlorine contaminated areas of Lake Ontario, Great Lakes (Table 3.14). For PCDDs, mean egg to whole-body ratios were 1.2, while for PCDFs ratios were 0.87. For total PCBs, the mean egg/whole body ratio on a lipid weight basis was 0.47. In the same study, biomagnification factors of 32 for TCDD and 93 for total PCBs (including non-dioxin-like congeners) were found from alewife (fish) to herring gull eggs and herring gulls whole body, respectively (Table 3.15).

**Table 3.14 Mean egg-whole tissue ratios for adult herring gulls from Lake Ontario (Braune and Norstrom, 1989)**

| | Egg/whole body | |
|---|---|---|
| **Congener** | **Lipid weight basis** | **Wet weight basis** |
| Total PCBs | 0.47 (± 0.19) | 0.34 |
| PCB 105 | 0.22 (± 0.08) | 0.20 |
| PCB 118 | 0.51 (± 0.19) | 0.38 |
| Mean PCDDs | 1.2 (± 0.33) | 0.81 |
| 2,3,7,8-TCDD | 0.81 (± 0.33) | 0.65 |
| 1,2,3,7,8-PnCDD | 0.88 (± 0.38) | 0.69 |
| 1,2,3,6,7,8-HxCDD | 1.1 (± 0.68) | 0.8 |
| 1,2,3,4,6,7,8-HpCDD | 1.6 (± 0.43) | 0.88 |
| OCDD | 1.6 (± 0.92) | 1.05 |
| Mean PCDFs | 0.87 (± 0.2) | 0.66 |
| 2,3,7,8-TCDF | NC | ND |
| 2,3,4,7,8-PnCDF | 0.89 (± 0.52) | 0.68 |
| 1,2,3,4,7,8-HxCDF<br>1,2,3,4,6,7-HxCDF | 0.88 (± 0.47) | 0.67 |
| 1,2,3,6,7,8-HxCDF | 0.84 (± 0.40) | 0.63 |

These authors found selective deposition in eggs of 1,2,3,4,6,7,8-HpCDD and OCDD compared to other congeners (i.e. higher ratios). No soil concentration data was provided, and so it cannot be determined whether this selectivity also occurred between soil and birds. For PCBs, the highest accumulation was seen in the more persistent higher chlorinated congeners with no adjacent unsubstituted positions followed by those unchlorinated only at the meta-ortho position.

**Table 3.15 Mean BMFs (wet weight basis) for selected congeners between adult herring gulls and fish (alewife) from Lake Ontario**

| Congener | BMF |
|---|---|
| Total PCBs | 93 (± 17) |
| PCB 105 | 102 (± 16) |
| PCB 118 | 80 (± 16) |
| 2,3,7,8-TCDD | 32 (± 9.2) |
| 1,2,3,7,8-PnCDD | 14 (± 3.4) |
| 1,2,3,6,7,8-HxCDD | 20 (± 2.5) |
| 1,2,3,4,6,7,8-HpCDD | NC |
| OCDD | NC |
| 2,3,7,8-TCDF | 1.3 (± 0.60) |
| 2,3,4,7,8-PnCDF | 6.6 (± 2.7) |
| 1,2,3,4,7,8-HxCDF; 1,2,3,4,6,7-HxCDF | NC |
| 1,2,3,6,7,8-HxCDF | NC |

In other studies, Lemmetyinen et al. (1982) found rates of elimination of PCBs via egg production of 45% body burden for female Arctic tern and 24% for herring gull. Tanabe et al. (1986) reported rates of elimination of only 4% PCB body burden for Adelie penguins after laying their annual clutch of two eggs. Elimination was thought to be dependent on the relative weights of the egg and mother.

Thomas and Anthony (1999) calculated BMFs for Great blue heron colonies at nesting sites in Washington and Oregon in areas of known dioxin contamination. BMFs were calculated at six sites from the geometric mean of residue concentrations in Great blue heron eggs divided by the geometric mean of residue concentrations in their prey items. Prey items were collected opportunistically at nesting sites. Compounds with the highest BMFs differed among sites for all contaminants except PCB 169. Of the PCBs, congener 126 and 169 had the highest BMFs, the former ranging between 7 and 150, and the latter between 9 and 32. PCB 77 had the lowest BMFs, ranging between 0.2 and 18. BMFs for TCDD ranged between 2 and 23, while BMFs for TCDF ranged between 0.2 and 3, with biomagnification occurring in three out of six sites. BMFs for OCDD ranged between 5 and 20. It was noted that the BMFs between prey and the whole body of birds are larger than the BMFs between prey and eggs. No sediment concentrations were provided in this paper so that bioaccumulation between sediment and eggs could not be calculated.

Studies have shown that free foraging chickens can take up PCDDs/PCDFs from soil and rapidly transfer them into eggs. The concentrations and congener profiles of PCDDs/PCDFs in the eggs of chickens appears to be related to the concentration in the soil on which they are raised (Stephens et al. 1990, 1995; Schuler et al., 1997).

Stephens et al., (1995) used chickens as a model for foraging animals to determine the bioavailability of PCDD/Fs in contaminated soil. BAFs were calculated from wet weight tissue concentrations and food concentration (soil conc. by fraction of soil in feed). Chickens were exposed to soil concentrations of 0.5 pg/g I-TEQ (control), 42 pg/g and 460 pg/g I-TEQ. Daily intake (soil formulated with feed) was estimated to be 0.3 (low) and 2.5 pg/g/day. Steady state concentrations were reached after 80 days. Tissue distribution was congener-dependent with 5 to 30% intake excreted in eggs, 7 to 54% deposited in adipose tissue, and less than 1% in the liver. On a fat weight basis, the highest concentrations were observed in the liver. The tetra-chlorinated chlorinated compounds showed the highest degree of bioaccumulation. Availability of tetra-chlorinated congeners was 70 to 80%, which declined to 10% for octa-chlorinated congeners. Bioaccumulation of the penta- through hepta-congeners was between these extremes.

Other terrestrial bioaccumulation studies have indicated a general tendency for the highest transfer of TCDD, 1,2,3,7,8-PeCDD and 2,3,4,7,8-PeCDF from soils to organisms and lowest transfer ratios for the higher chlorinated PCDDs/PCDFs and also for 2,3,7,8-TCDF. Schuler et al., (1997) determined bioaccumulation from sediment to eggs using transfer ratios (Table 3.16) based on ratios of the concentrations in egg on a lipid weight basis, and concentration in soil on a dry weight basis.

**Table 3.16 Transfer ratios for PCDD/Fs from soil to eggs**

| Congener | Soil to egg transfer ratio (BSAF) |
|---|---|
| 2,3,7,8–TCDD | 1.2 |
| 1,2,3,7,8–PeCDD | 2.4 |
| 1,2,3,4,7,8–HxCDD | 1.5 |
| 1,2,3,6,7,8–HxCDD | 1.6 |
| 1,2,3,7,8,9–HxCDD | 0.8 |
| 1,2,3,4,6,7,8–HpCDD | 0.4 |
| OCDD | 0.1 |
| 2,3,7,8–TCDF | 3.3 |
| 1,2,3,7,8–PeCDF | 4.4 |
| 2,3,4,7,8–PeCDF | 0.8 |
| 1,2,3,4,7,8–HxCDF | 0.9 |
| 1,2,3,6,7,8–HxCDF | 1.0 |
| 2,3,4,6,7,8–HxCDF | 0.6 |
| 1,2,3,7,8,9–HxCDF | 0.1 |
| 1,2,3,4,6,7,8–HpCDF | 0.2 |
| 1,2,3,4,7,8,9–HpCDF | 0.1 |
| OCDF | 0.1 |

No information was provided on the organic carbon content in the soils, so these data cannot be converted to organic carbon normalised BSAFs. Results showed the transfer of individual congeners decreases over more than one order of magnitude with increasing chlorination from PeCDD/PeCDF to OCDD/OCDF (Table 3.16).

In mammals, the transfer of dioxins from mother to offspring during lactation is an important route of exposure to newborn aquatic mammals. However, only limited data is available on the amount of dioxins transferred from mother to young in wildlife. The amount transferred varies, and depends on the fat content of the milk, the duration of lactation, and the birth order. Dolphins, porpoises, and whales produce the most lipid-rich milk reported in mammals. For example, gray whale milk contains up to 53% fat, beluga milk contains 45% fat, and dolphin milk ranges between 30 and 35% fat (Colborn and Smolen, 1996 and references therein).

Colborn and Smolen (1996) review several studies examining maternal transfer in marine mammals. These studies reported 98% transfer of the maternal body burden of dioxins to grey seal (*Halichoerus grypus*) pups. The placental transfer of dioxins is much lower than in milk, with about 1% in grey seals and 2% in Weddell seals (*Leptonychotes weddelli*). Another study estimated that a striped dolphin's first calf may receive up to 80% of the maternal body burden of dioxins, with succeeding calves receiving about a quarter of that amount. Studies reviewed in (WHO, 1993 and references therein) reported that female grey seals excrete 15% of their body burden of PCB via lactation. Striped dolphins transferred between 72 and 98% of their body burden to offspring. This large transfer was because of the high lipid content in dolphin milk.

McLachlan et al. (1990) studied the transfer of dioxins from grass to milk by determining the PCDDs/PCDFs fluxes out of the cow into milk, calculated as percentage input. Their results showed transfer ratios of between 0.35 and 0.04 for PCDD congeners, and 0.25 to 0.01 for OCDFs congeners. Fürst et al. (1993b) investigated PCDDs/PCDFs levels in cow's milk in relation to their levels in grass and soil. Their results indicated that the transfer pathway from air to grass to cow is more important than the pathway from soil to grass to cow. The transfer of PCDDs/PCDFs between grass and cow's milk differs significantly depending on congeners. The highest transfer was seen for 2,3,7,8-TCDD, while the lowest was for OCDD, with a difference factor of almost 40 between these two congeners. The relative ratio of the factors determined for each congener was very similar irrespective of the dioxin levels in the grass at the different sampling sites.

## 3.8 Summary and conclusions

This report assesses the levels of exposure experienced by Australian native fauna to 29 dioxin-like congeners measured in the NDP fauna survey. Exposure to dioxins is determined initially from tissue concentrations (body burden), which represent the amount of dioxins that have accumulated in an organism over time from all exposure sources. Toxic Equivalence methodology is used to translate congener concentrations in organisms to a single TEQ value. Exposure is further characterised by determining the

bioaccumulation potential of individual dioxin-like congeners in organism from soils and sediments (BSAF).

Dioxins were found in soils, sediments and fauna in all geographical environments and land use types, although the levels were highly variable, particularly in fauna. The dioxin levels in soil were on average highest in industrial and urban environments. The dioxin levels in sediment were highest in urban and industrial estuarine environments. The dioxin levels in fauna reflect the animal's position in the food chain, with high trophic level aquatic and terrestrial organisms having the highest exposure relative to other organisms in the same environment. On average, raptors had the highest TEQs of all the fauna sampled.

The congener profiles in soil and sediment samples were dominated by the higher chlorinated PCDDs/PCDFs, in particular OCDD. The profiles for PCBs were generally dominated by PCB congeners 118, PCB 105, PCB 156 and PCB 77. The congener profiles in fauna were similar to those in soil and sediment for both PCDD/Fs and PCBs.

Concentration ratio (or enrichment) between soil/sediment and fauna tissue (BSAFs) were determined based on a limited number of measured tissue concentrations, and soil and sediment concentrations. The BSAFs differed for each congener, with the tetra through hexa-chlorinated congeners accumulating the most, and the more abundant higher chlorinated PCDD/PCDFs accumulating the least in organisms relative to soil and sediment. The BSAFs also varied across sites, species and trophic level. Biomagnification was greatest in raptors and in dolphins. It appears, based on limited data, that for raptors, PCDD/PCDFs and PCBs were biomagnified, while for aquatic mammals, only PCBs were biomagnified to any extent.

Bioaccumulation is influenced by a large number of variables, both dependent on characteristics of the exposure medium and on the physiological and metabolic characteristic of the organism. Thus, variations in bioaccumulation factors among individual fauna and between sites and ecosystems are not surprising. This variability results in inherent uncertainty in the estimation and interpretation of bioaccumulation. With the information currently available, it would appear unlikely that a universal relationship between sediment/soil and tissue concentrations can be applied with confidence between different sites, ecosystems and species.

To predict body burden in organisms from the BSAF and soil/sediment concentrations, the approach used in this risk assessment is to adopt the average and maximum BSAFs for each class of organism and for each congener. Adopting the upper limit of accumulation will provide a more conservative estimate of the actual body burden an animal may acquire from the exposure levels encountered in the Australian aquatic and terrestrial environment.

It is acknowledged, however, that this approach is associated with large uncertainties. Sources of the uncertainty include the small number of samples (both soil/sediment and fauna), lack of information on the characteristics of the organisms (e.g. age, gender) sampled, the large variability in exposure concentrations among abiotic and biotic samples, and the difficulties in determining the average exposure under field situations. To avoid repetition, these exposure predictions will be presented in Chapter 4, where they will be compared with the toxicity reference values (TRV) derived from ecotoxicological studies.

# 4 Risk Characterisation

## 4.1 Introduction

This chapter estimates the likelihood and severity of risk to native aquatic and terrestrial organisms exposed to dioxins at ambient levels found in the Australian environment. The risk is assessed through the integration and development of information previously presented in chapters on the exposure and effects assessments. This chapter also describes the sources and types of uncertainties associated with the risk assessment. There are a number of sources of uncertainty in all risk assessments. These uncertainties arise at all stages of the risk assessment and have implications for how much confidence can be placed in the risk estimation. It is therefore important to identify and describe the sources and types of uncertainties associated with the risk assessment.

A tiered approach is initially adopted to estimate risk, involving an initial screening level characterisation. This is followed by higher tiered risk characterisation, if the screening level assessment indicates that further clarification is required.

The tier one risk characterisation involves a simple risk determination using the quotient method. In this method conservative criteria are adopted to derive a risk quotient (Q), whereby the lowest observed effect level for the most sensitive species tested in ecotoxicology studies is compared to the highest exposure concentration found in the exposure assessment (Klaine et al., 1996). A $Q<1$ implies a low risk, while a $Q>1$ implies a potential risk.

The quotient method is a simple and efficient way of screening out the low-risk groups of organisms from the high-risk groups, allowing emphasis to be placed on those organisms most at risk. However, the quotient method does not provide an incremental measure of risk whereby the magnitude of the likely impacts can be ascertained. In other words, a Q result of two does not mean that the risk is twice as high as a Q result of one. When a potential risk is indicated by the quotient method, further refinement is required using a higher tiered risk characterisation method to determine the magnitude and likelihood of the risk.

For higher tiered risk characterisation, the cumulative probability distributions of the measured body burden in organisms (i.e. the exposure) are plotted against the cumulative probability distributions of the ecological effects (i.e. toxicology data). By comparing the distributions of exposure with effects, the likelihood of the risk to individual animals and to different segments of wildlife populations are described. In this method, variability in exposure is used to predict changes in the magnitude and likelihood of effects, while the variability in species-sensitivity to effects is used to estimate risks to average or sensitive population members.

A further consideration in the higher tiered risk assessment is to combine information from a variety of sources such as epidemiology, biology, and ecology, to determine whether the risk estimates and the conclusions of the risk assessment are reasonable and are consistent with, and supported by, the other available lines of evidence.

## 4.2 Risk characterisation methods

### 4.2.1 Risk quotients

To characterise risk to organisms using the risk quotient (Q) method, risk is expressed as an exposure concentration point estimate divided by an effects concentration point estimate, termed the exposure reference value and the toxicity reference values, respectively:

$$\text{Risk Estimate (Q)} = \frac{\text{Exposure Reference Value}}{\text{Toxicity Reference Value}}$$

Q values greater than 1.0 signify the likelihood or potential for adverse effects to occur, while Q values less than one imply no hazard to organisms and no further risk assessment.

Conservative (worst-case) criteria are used for screening purposes to derive the risk quotient, whereby the lowest adverse effect level for the most sensitive species tested in laboratory toxicology studies is selected as the TRV for comparison with the highest exposure concentration. The most conservative estimate of exposure is the highest dioxin residue concentration found in the tissue of an individual organism from a particular class (i.e. fish, birds, mammals) of organisms. This value is used as the exposure reference value to represent the risk to all wildlife in that particular class.

The most conservative effect level determined in laboratory studies with dioxins is the no-observed-adverse-effects level (NOAEL). The NOAEL describes the highest level at which no toxic effects are observed, and this endpoint is preferred over the LOAEL, which is less conservative, and describes the lowest level at which toxic effects occurred.

The most sensitive response with ecological relevance is also an important criterion defining the TRV. Reproductive and developmental effects are among the most sensitive responses seen in fish, birds and mammals when exposed to low doses of dioxins under chronic conditions.

Thus, the risk determination will adopt the NOAEL for reproductive and developmental effects as the TRVs, based on the most sensitive species within each broad class of organism (i.e. fish, birds, mammals). It is assumed that the NOAEL for the most sensitive test species within a class of organisms should also protect other related species in the environment, including Australian native wildlife. TRVs determined from toxicology studies with laboratory animals are used as surrogates for extrapolation to Australian native species to determine the level of risk to wildlife. Field studies may be used to derive TRVs when no laboratory data are available. Extrapolation is necessary because of a lack of toxicity data for wildlife in general, and for Australian native wildlife in particular.

Exposure and toxicity reference values used to derive Q must be expressed in the same units. The most sensitive TRVs for dioxins are expressed in terms of the TCDD Toxic Equivalent Concentration (TCDD TEQs) in the tissue of biota that resulted in the observed effect. For studies using congeners other than 2,3,7,8-TCDD (where TEF = 1), the endpoints are multiplied by their respective TEFs to derive a TCDD TEQ. Similarly, the exposure reference values are expressed in terms of TCDD TEQs, which are determined from the sum of the concentration of all of the congeners (i.e. PCDDs, PCDFs and PCBs) measured in the whole organism of the target species, multiplied by the respective congener and species-specific TEFs.

### 4.2.2 Probability distribution curves

The cumulative probability distribution of the measured tissue concentrations in organisms is plotted against the distribution of available ecological effects, also based on tissue concentrations, for higher tiered risk characterisation. Separate cumulative probability distributions are derived for single classes of organisms (e.g. fish and birds) for which tier one assessment indicates a potential risk (Q >1). Cumulative probability distributions may also be used for comparative purposes and to visually present data in tier-one risk determinations.

To derive the distribution curves, the available effects and exposure concentrations (expressed as total TEQs) are ranked from highest to lowest. Ranks are then converted to proportions using the Hazen equation:

$$\text{Proportion} = (I - 0.5)/n \text{ X } 100$$

Where I = the rank and n = the number of data points. This formula compensates for small data sets (Solomon, 1999). The resulting value represent the proportion of individuals (or species) with a body burden, or effective concentration (in the case of toxicity data), less than or equal to that particular concentration.

For mammals, no cumulative probability distributions are derived for effects, because available toxicity data based on body burden are not sufficient to support this approach. Instead threshold ranges are used to compare with the exposure. For birds, and fish, the TRVs used to the derive effects distribution curves, also called species sensitivity distributions (SSDs), are discussed in the relevant sections that follow for each different classes of organisms.

### 4.2.3 Expressing exposure and effects in comparable units

#### Converting maternal body burden to egg burden

For fish and birds, the most sensitive TRVs are expressed as contaminant concentrations in eggs rather than concentrations in whole fish or bird. This reflects the greater sensitivity of early life stage fish (eggs and sac fry) and bird embryos to the toxic effects of dioxins when compared to hatchings, juveniles and adults. No egg concentrations are available from which to derive exposure reference values; consequently, measured concentrations in fish

and birds must first be converted to concentrations in eggs so that risk can be determined for the more sensitive early life stages.

Limited studies are available examining the amount of dioxin that is transferred from the maternal tissue to eggs. Russell et al. (1999) indicate that for oviparous (egg producing) organisms, lipid adjusted chemical concentrations in eggs and maternal tissue are fairly close, and typically are within a factor of one or two for lipid soluble organic chemicals. At equilibrium, the relationship between the chemical concentration in the eggs and maternal tissue reflects the difference in their lipid concentrations.

This relationship is expressed as:

$$EMR_L = C_{EL}/C_{ML}$$

Where $EMR_L$ = lipid normalised egg-to-muscle tissue ratio

$C_{EL}$ = lipid normalised concentration in eggs

$C_{ML}$ = lipid normalised concentration in maternal tissue

The transfer model assumes that chemical transport from the mother to the eggs follows a set of passive (non-energy consuming) transport processes resulting in chemical equilibrium between the mother's tissue and the eggs. Other assumptions are that transfer involves relatively rapid and homogeneous distribution of lipoproteins between the mother and the eggs; resulting in chemical levels in the egg that reflect those in maternal lipoproteins. Metabolic transformation of the contaminants in eggs is expected to be low as critical enzyme systems are poorly developed in embryonic tissue.

Available data for fish indicate the ratio between the lipid adjusted chemical concentrations in fish eggs and maternal tissue is typically within a factor of 1.0. This means that during fish development, the embryos would be exposed to the same internal lipid normalised concentrations as the maternal organism from which the eggs originated. For birds the EMR is nearer to 0.6, although this is based on a very small data set. For example, a lipid normalised EMR of 0.65 was reported for total PCB in the Adelie penguin (Tanabe et al., 1986). Lipid normalised EMRs of 0.5 and 0.67 were reported for PCB in herring gulls and Arctic terns, respectively (Lemmetyinen et al., 1982), while mean lipid normalised EMRs of 1.2 for PCDDs, 0.87 for PCDFs, and 0.47 for PCBs were reported in herring gull eggs from Lake Ontario (Braune and Norstrum, 1989).

For the purpose of this risk assessment, it is assumed that the lipid normalised EMR is 1.0 for PCDDs, PCDFs and PCBs between the maternal fish tissue and fish eggs. For birds, it is assumed that the lipid normalised EMR for PCDDs is 1.5, for PCDFs is 1.0, and for PCBs is 0.7 between the maternal bird tissue and bird eggs. These EMRs are the most conservative estimates of maternal transfer available in the literature, and adopt the upper confidence limits (Braune and Norstrum, 1989).

**Converting soil concentrations to body burden**

Soil and sediment data are used to predict tissue concentrations in fish, birds and aquatic mammals by way of the measured BSAFs and using the measured congener levels in soil and sediment. These data are used to develop cumulative probability distributions for

exposure to provide additional exposure information. It is emphasised that these prediction are experimental in nature because of the uncertainties in the BSAFs and in the applicability of the equations (see below). The data are used largely to explore the feasibility of using soil and sediment data to predict tissue levels in organisms.

The ability to predict the maximum steady-state body burden that could be reached in top predators under any given exposure condition would enable risk assessors to better predict maximum possible risk to these organisms when no tissue data is available. This information would also be useful to help determine safe threshold levels for setting soil and sediment quality guidelines. Guidelines for acceptable threshold levels of chemicals in soil and sediment have been set in some countries for specific purposes such as for agricultural use or for the protection of fish or wildlife. However, setting appropriate threshold levels for bioacumulating chemicals such as dioxins is a notoriously difficult and complex task.

Point concentrations in soil and sediment cannot be related directly to the concentrations in organisms because the pattern of congeners varies between soil and sediment and in organisms of different species and at different trophic levels. These variations in congener patterns result from the differences between individual congeners in bioavailability, uptake and elimination in the digestive tract, and bioaccumulation through the food chain.

In order to relate dioxin exposure concentrations in abiotic media to exposure in organisms, the soil and sediment concentrations must first be converted to concentrations in either the tissues of the organisms, or their food, prior to applying TEFs for calculating TEQs (US EPA, 2003). This is achieved by application of appropriate congener and species-specific biota-sediment accumulation factors (BSAFs). The BSAFs provide a measure of the relative concentration of each congener in abiotic compared to biotic media. For some organisms (i.e. low trophic level organisms) and congeners (i.e. low bioaccumulation potential), the levels in the soil will be higher than those in the organism's tissue, while for other organisms (high trophic level organisms) and congeners (i.e. high bioaccumulation potential), the levels will be higher in the organism than in the soil.

For this risk assessment, the mean BSAF determined from measured concentrations in biotic and abiotic media (Chapter 3) will be used to predict the potential tissue concentrations that an animal may acquire for a given soil or sediment exposure level.

Equations used to relate the dioxin concentrations in abiotic media to the concentrations in the tissue of organisms, in terms of Toxic Equivalents (TEQ), are adapted for this risk assessment from US EPA (2001) and US EPA (2003). These documents were available only in a draft form at the time this ERA was conducted, and the models presented have not yet undergone field validation.

The equations are generalised and summarised below.

Fish egg concentrations of PCDDs, PCDFs, and PCBs are related to the surficial sediment concentrations as:

$$TEQ_{fish\ wet\ weight} = \sum_{i}^{n} 1\ [(C_{oc})_i\ (f_{fl})\ (EMR_L)\ (BSAF_{fish})_i\ (TEF_{fish})_i]$$

Where i represents the $i_{th}$ congener, $(C_{oc})_i$ is the organic carbon-normalised concentration of each congener in the surficial sediments, $f_{fl}$ is the fractional lipid composition of fish eggs (7%), $EMR_L$ is the egg-to-muscle lipid normalised ratio for PCDD, PCDF and PCBs transfer between the maternal tissue and eggs. The $BSAF_{fish}$ is the congener-specific biota-sediment accumulation factor and is normalised for both tissue lipid and the organic carbon content in the sediment. The fish-specific TEFs ($TEF_{fish}$) for each congener are used to make the final conversion to a wet weight fish dioxin toxic equivalent ($TEQ_{fish\ ww}$).

The contaminants in fish eggs are assumed to be in equilibrium such that the relative concentrations of congeners are the same in both tissues, and the absolute concentrations are directly proportional to the fractional composition of the lipid.

The formula used to relate the surficial soil concentrations of PCDDs, PCDFs, and PCBs to TEQ in the eggs of raptors is:

$$TEQ_{bird\ egg\ ww} = \sum_{i}^{n} 1\ [(C_{oc})_i\ (f_{fl})\ (BSAF_{bird})_i\ (EMR_L)\ (TEF_{bird})_i]$$

Where, $C_{oc}$ is the organic carbon normalised soil concentration, $f_{fl}$ is the fractional lipid composition of bird eggs, $BSAF_{bird}$ is the biota-soil accumulation factor measured in a bird of prey, $EMR_L$ is the egg-to-muscle lipid normalised ratio for PCDD, PCDF and PCBs transfer between the maternal tissue and bird eggs, (the fractional lipid composition of the bird eggs is assumed to be 8%), and $TEF_{bird}$ is the bird-specific TEF. The resultant sum of the congeners is the total dioxin-like potency expected in the bird eggs on a wet weight basis.

The formula that relates the surficial sediment concentrations of PCDDs, PCDFs, and PCBs to TEQ in mammals is:

$$TEQ_{mammal\ lipid} = \sum_{i}^{n} 1\ [(C_{oc})_i\ (BSAF_{mammal})_i\ (TEF_{mammal})_i]$$

Where the $(BSAF_{mammal,\ l})_i$ is the congener-specific BSAF determined for mammals and $TEF_{mammal}$ is the mammal-specific TEF. Mammalian TRVs are expressed on a lipid weight basis, and hence, the term for fractional lipid composition is omitted from the formula. The resultant sum of the congeners is the total dioxin-like potency expected in the mammal on a lipid weight basis.

## 4.3 Risk to bivalves

There are only limited data on the toxicity of dioxins to invertebrates, and no TRVs are available for bivalves. The available studies indicate that invertebrates (which includes a mollusc i.e. snail) are not sensitive to the toxic effects of dioxins. The observed lack of sensitivity of invertebrates is consistent with the view that the Ah receptor is not present in these organisms. However, invertebrates have the ability to accumulate relatively high concentrations of dioxins, and are therefore potential sources of exposure to higher organisms consuming them for food.

## 4.4 Risk to fish

### 4.4.1 Most sensitive TRV for fish

Fish species vary in their sensitivity to dioxins. The salmonid fish are the most sensitive class of fish to the toxic effects of dioxins, and the most sensitive species is the lake trout. Early life stage fish are significantly more sensitive than adults. The NOAEL for sac fry mortality in different freshwater fish following embryo exposure range between 34 pg TCDD/g and 1,190 pg TCDD/g, in fish eggs (Chapter 2, Table 2.5). The $LD_{50}$ for early life stage lake trout in terms of sac fry mortality is 65 pg TCDD/g, while the NOAEL is 34 pg TCDD/g wet weight egg. Thus, a threshold egg burden of 30 pg TEQ/g (using fish TEFs) in fish eggs is selected as the TRV to characterise the level of risk to fish.

It is reasonable to assume that a TRV slightly lower than the NOAEL for the most sensitive freshwater fish species based on sac fry mortality should be protective of other fish species in the environment. For example, adult female lake trout are able to grow and spawn successfully at dietary exposure levels of TCDD that cause 100% mortality in sac fry exposed through maternal transfer (Johnson et al., 1998). Thus, a TRV protective of sensitive sac fry, will be protective of the less sensitive adult and juvenile fish.

Limited data are available for dioxin-like toxicity in marine and estuarine freshwater fish. However, saltwater species for which data are available are less sensitive to dioxins than many freshwater species. Thus, a TRV protecting sensitive freshwater fish species should be sufficient to screen the level of risk to estuarine and coastal marine fish species.

An NOAEL based on sac fry mortality for the most sensitive freshwater fish species should also cover a range of other adverse effects induced by exposure to dioxins, because for fish there tends to be significant overlap between the types and level of responses. The most severe effect on the spectrum of toxic responses observed in fish following exposure to dioxins is death, while the least severe responses are biochemical changes such as increased enzyme induction, which manifest on a cellular level and are not necessarily associated with obvious outward toxic responses. The ecological relevance of biochemical changes resulting in enzyme induction can be difficult to demonstrate. For many fish species, the difference between the concentration having minimal effects and the concentration having adverse effects is quite small, particularly in early life stages. For example, in early life stage lake trout, the dose-response relationship for TCDD induced cytochrome P4501A expression in vascular endothelial cells, parallels that for TCDD-induced sac fry mortality and associated cardiovascular dysfunction (Guiney et al., 1997). For Brook trout, the concentration required to induce cytochrome P4501A expression is slightly lower than the concentration required for 10% mortality in sac fry (Johnson et al., 1998).

### 4.4.2 Exposure reference values in fish

Tissue concentrations are available for 23 individual mature fish comprising 12 different fish species (Müller et al., 2003 b). Tissue concentrations, representing our exposure reference value, range from 0.7 pg TEQ/g lipid to about 60 pg TEQ/g lipid, using fish specific TEFs and the full LOD (Chapter 3, Table 3.10).

In the wild, fish embryos are most likely to be exposed to dioxin through maternal transfer from the adult female to the developing eggs rather than through waterborne or sediment exposure. If we assume that the levels measured in whole fish are representative of the maternal body burden in mature female fish, and the transfer ratio (EMR) to eggs is 1:1, the egg loads also range from 0.7 pg TEQ/g lipid to about 60 pg TEQ/g lipid per egg.

Fish TRVs, however, are typically reported on a whole egg wet weight basis rather than on a lipid-normalised basis. Consequently, predicted concentrations in the fish need to be converted to whole egg wet weights. To make this conversion, it is assumed that fish eggs have a lipid content of 7% of whole egg (US EPA, 2003). The results of the conversion are provided in Table 4.1, along with the risk quotient (Q). The Q values are derived from the predicted concentration in fish eggs divided by the most sensitive species TRV (30 pg/g).

**Table 4.1 Estimated TEQs in fish eggs and resulting risk quotients (Q)**

| | | pg TEQ/g | | |
|---|---|---|---|---|
| **Species** | **Location** | **Maternal (lw)** | **Egg (ww)** | **Q** |
| Barramundi | Gulf of Carpentaria | 60 | 4.26 | 0.145 |
| Golden Perch | Coopers Creek | 18 | 1.26 | 0.042 |
| King George Whiting | Port Jackson | 10.7 | 0.75 | 0.025 |
| River Cobbler | Albany Region | 10 | 0.71 | 0.024 |
| Sand Whiting | Moreton Bay | 9.5 | 0.67 | 0.022 |
| Barramundi | Darwin Region | 6.5 | 0.46 | 0.015 |
| Bream | Moreton Bay | 6.1 | 0.43 | 0.014 |
| Barramundi | Cairns Region | 5.4 | 0.38 | 0.013 |
| Flathead | Moreton Bay | 6.1 | 0.38 | 0.013 |
| King George Whiting | Melbourne Region | 5.6 | 0.40 | 0.013 |
| Flathead | Melbourne Region | 4.7 | 0.33 | 0.011 |
| Flathead | Albany Region | 4 | 0.27 | $9.0 \times 10^{-3}$ |
| Short-finned Eel | Latrobe River | 2.8 | 0.19 | $6.3 \times 10^{-3}$ |
| Golden Perch | Murray River | 2.8 | 0.19 | $6.3 \times 10^{-3}$ |
| Green Backed Flounder | Melbourne Region | 2.1 | 0.14 | $4.7 \times 10^{-3}$ |
| Barramundi | Roebuck Bay | 1.9 | 0.135 | $4.5 \times 10^{-3}$ |
| Flathead | Gippsland Lakes | 1.4 | 0.10 | $3.3 \times 10^{-3}$ |
| Flathead | Port Jackson | 1.5 | 0.09 | $3.0 \times 10^{-3}$ |
| Australian Salmon | Hobart - Storm Bay | 1.3 | 0.09 | $3.0 \times 10^{-3}$ |
| Flathead | Triabunna Region | 1.2 | 0.08 | $2.7 \times 10^{-3}$ |

| | | | | |
|---|---|---|---|---|
| Bream | Port Jackson | 0.7 | 0.05 | $1.7 \times 10^{-3}$ |
| Flathead | Adelaide Region | 0.7 | 0.05 | $1.7 \times 10^{-3}$ |
| Garfish | Adelaide Region | 0.7 | 0.05 | $1.7 \times 10^{-3}$ |

Egg concentrations fall below the threshold value, resulting in the Q values for all fish being significantly less than one. Therefore, it is reasonable to assume, based on the available data, a low level of risk to early-life stage fish at the ambient exposure levels found in the Australian aquatic environment. A low risk is also assumed for reduced reproduction and adverse effects on recruitment of fish populations. The risk to adult and juvenile fish from exposure to dioxins at ambient levels is even lower than for early-life stage fish.

The number of fish species and the sample size for which tissue concentrations are available is very small. Consequently, it is not known how representative these fish are of entire fish populations and of different fish species. Most of the fish sampled in the survey are pelagic fish, consumed by humans, and may not reflect the diversity of fish in the Australian aquatic environment in terms of species, trophic level, habitat type, and exposure conditions. Nevertheless, the available species do occupy a range of habitat types and consume different food items. These include open-ocean, estuarine, freshwater and coastal fish, and with some bottom dwelling species also represented. A low risk is indicated for all of these species.

### 4.4.3 Predicted exposure from sediment levels

Figure 4.1 shows the TEQs in fish eggs (wet weight egg), plotted against the species sensitivity distributions. The egg TEQs include those calculated from measured dioxin levels in fish tissue (open circles, data from Table 4.1), and those predicted from the dioxin levels in estuarine sediment and the average and maximum BSAFs for fish. The species sensitivity distribution curves are derived from the NOAEL and LOAEL for sac fry growth and mortality in various freshwater fish exposed to TCDD in laboratory studies (i.e. Chapter 2, Table 2.5).

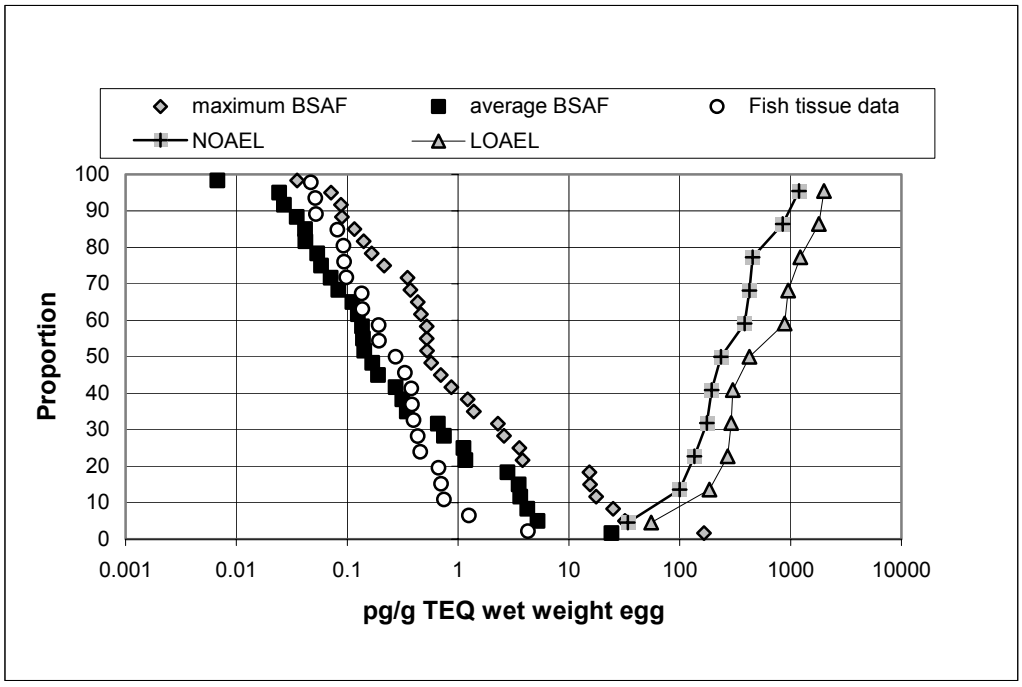



**Figure 4.1 Species sensitivity and exposure distribution curves of TEQs in fish eggs**

Compared to the TEQs derived from measured tissue levels, both the average and maximum BSAFs overpredict the egg TEQs when the sediment concentrations increase above a certain level. However, even with these overpredictions, less than 7% (2 of 30) of predicted egg TEQs are equal to, or greater than, the NOAEL for the most sensitive fish species, while 3% (1 of 30) of predicted TEQs are greater than the LOAEL, using the maximum (worst-case) BSAF and using all of the available data for estuarine sediments. Note that the egg concentrations are above the threshold TRV, when the distribution curves on the left (i.e. TEQs in eggs) overlap the distribution curves on the right (i.e. TRVs for different species). These results suggest a low risk to estuarine fish exposed to dioxins at ambient environmental levels. The dioxin levels found in marine and freshwater sediments were significantly lower than levels found in estuarine sediments in urban and industrial regions, and as such, the risk to fish living in these environments is expected to be even lower than for estuarine fish.

The data point lying above the threshold TRVs is derived from a sediment sample collected from the lower Parramatta River in the vicinity of Homebush Bay, which is a recognised hot spot for dioxins and other chemical contamination resulting from chemical manufacturing in the area from 1928 through to 1986. The site is about to undergo remediation.

## 4.5 Risk to birds

### 4.5.1 Toxicity reference values

There are at present no screening toxicity benchmarks for dioxins in birds. The literature on avian toxicity encompasses both field and laboratory data. These data indicate bird embryos are significantly more sensitive than adults and hatchlings to the toxic effects of dioxins, although there is considerable variability in sensitivity among avian species. For some bird species, field effects appear to be more sensitive than laboratory effects. Caution is required, however, when interpreting field measured toxicity because the data lacks standardisation, particularly in terms of analytical methods and reported endpoints, making it incompatible and difficult to compare among studies and between species. Interpretation of field exposure is also complicated by the possible presence of other contaminants besides dioxins, and by the potential impact of other non-chemical stressors on the survival and development of birds. Thus, for many of the field studies, reliable TRVs cannot easily be determined.

A comparison of the laboratory and field data on avian embryo toxicity is provided in Table 4.2. In laboratory studies, galliformes (domestic chicken, pheasant, quail, grouse, turkey etc) appear to be the most sensitive order of bird, with the domestic chicken by far the most sensitive avian species tested. The lowest reported LOAEL for embryo toxicity and developmental effects (i.e. cardiac malformations) in chickens is 9 pg/g egg, fresh weight. The next most sensitive species of galliforme is the ring-necked pheasant, with an NOAEL for developmental impairment in embryos of 100 pg/g egg, which is approximately 10-100 times less sensitive than the chicken (Nosek et al., 1993; 1992a). The turkey (*Meleagris gallapavo*) has an NOAEL for developmental impairment in embryos of 10,000 pg/g egg (Brunström and Lund, 1988).

**Table 4.2 TRVs derived from field and laboratory studies with birds exposed to dioxin-like compounds**

| Species | Study type | Concentration ww egg | Effect level/response |
|---|---|---|---|
| Domestic chicken | Laboratory | 9 pg TEQ/g | LOAEL malformations and edema |
| Ring-necked pheasant | Laboratory | 100 pg TEQ/g | NOAEL embryo mortality |
| American kestrel | Laboratory | 230 pg TEQ/g | NOAEL malformations and edema |
| Double-crested cormorants | Laboratory | 1000 pg TEQ/g<br>4000 pg TEQ/g | NOAEL embryo mortality<br>LD50 for embryo mortality |
| Common tern | Laboratory | 44000 pg TEQ/g | LOAEL malformations and edema |
| Wood duck | Field | 20 pg TEQ/g | LOAEL egg hatchability and fledgling numbers |
| Osprey | Field | 136 pg TEQ/g | NOAEL for embryo mortality |
| Bald eagles | Field | 100 pg TEQ/g | NOAEL for hepatic CYP1A |

| | | | |
|---|---|---|---|
| | | 210 pg TEQ/g | induction<br>LOAEL for embryo survival |
| Double-crested cormorants | Field | 350 pg TEQ/g | LOAEL for embryo mortality |
| Great blue heron | Field | 17.6 pg PCDD/F/g<br>100 - 278 pg TEQ/g | NOAEL<br>Subcutaneous edema, fluid in the brain cavity, blocked cloaca intestinal uric acid accumulation, unresorbed yolk sac |

In field studies, anseriformes (ducks, swans, geese etc.) appear to be relatively sensitive to dioxins. In wood ducks, field derived TEQs of 20-50 pg/g wet weight egg are associated with a significant reduction in egg hatchability and fledgling numbers (White and Seginak, 1994). However, in laboratory studies anseriformes appear to be less sensitive than some other species. The geometric mean NOAEL for the mallard duck (*Anas platyrhynchos*) derived in laboratory experiments is 3500 pg TEQ/g, while for greylag geese (*Anser anser*), the geometric mean NOAEL is 50,000 pg TEQ/g (Suter, 2003 and references therein).

For falconiforme (hawks, eagles, osprey) species, reported NOAEL levels are at least 136 pg/g TEQ for embryo survival in wild ospreys (Woodford et al., 1998). For bald eagles, the NOAEL, using hepatic CYP1A induction as a biomarker, is determined to be 100 pg TEQ/g egg (wet weight), and the LOAEL for embryo survival is around 210 pg TEQ/g (Elliot et al., 1996) (Ahlborg et al., 1994, TEFs) on a whole egg wet weight basis.

Only one laboratory study is available for a falconiforme, the American kestrel. In this study, an NOAEL for embryo mortality of 230 pg TCDD TEQ/g was established using PCB 126 and PCB 77 (Hoffman et al., 1998). These data suggest falconiformes are also relatively sensitive to the toxic effects of dioxins compared to many other bird species.

Other wading water birds appear to exhibit low to intermediate sensitivity to the toxic effects of dioxins relative to other birds. For double-crested cormorants, the median $LD_{50}$ for TCDD based on embryo mortality in laboratory studies is 4000 pg/g egg (Powell et al., 1998). TEQs between 200-300 pg/g (based on H4IIE-derived TEQs) have been determined for embryo mortality in other studies with the double-crested cormorants. Developmental abnormalities, including beak deformities, have been associated with average concentrations in the eggs of 1300 pg TEQ/g, 20 pg TCDD/g, and 3600 pg PCB 126/g. Egg mortalities of between 8 and 39%, were associated with TEQs ranges between 35 and 344 pg/g, with planar PCBs accounting for most of the dioxin equivalents (Tillitt et al., 1992; Powell et al., 1998, and references therein).

In great blue heron chicks, mean field TEQs of 472 pg/g ($WHO_{98}$ avian TEFs) had some effect on biochemical and morphological parameters, but did not reduce the survival of embryos. Mean TCDD between 135 pg/g and 211 pg/g, wet weight, were associated with reductions in plasma calcium concentration, yolk-free body weight, tibia length, wet, dry, and ash weights, beak length, kidney weight, and stomach weight. Fewer down follicles were also observed on the heads of these chicks. Gross abnormalities observed in the

chicks included subcutaneous edema of the neck, legs, and abdomen, and one case of a crossed bill (Hart et al., 1991). In other studies with great blue heron, mean field TEQ levels between 100 and 278 pg/g in eggs were associated with gross abnormalities in hatchlings included subcutaneous edema and fluid in the brain cavity. TCDD concentrations were inversely related to body, yolk-free body, stomach, and intestine weight, tibia wet, dry, and ash weight, and tibia length. Hepatic EROD activity was directly related to TCDD concentration (Sanderson et al., 1994).

Shore birds such as gulls, terns, oyster catchers, plovers etc. appear to be less sensitive to dioxins than galliformes, anseriformes, falconiforme and other water birds for which data is available. In field collected common tern eggs hatched in the laboratory, the LOAEL for biochemical and decreased bursa weight was 25 µg TEQ/kg, liver lipid. This level did not have any effect on growth and development of chicks (Bosveld et al., 2000). In Forster's tern, a total mean TEQ of 2,175 µg/kg, wet weight, whole egg (derived for TCDD and PCBs using older TEF systems) was associated with a 50% reduction in hatching success in field collected eggs incubated in the laboratory (Elliott et al., 2001). Laboratory geometric mean NOAELs for Black-headed gull (*Larus ridibundus*) and herring gull (*Larus argentatus*) are both 50,000 pg TEQ/g (Suter, 2003 and references therein).

Passerines (perching birds) appear to be the least sensitive order of birds, based on the very limited data available. In tree swallows, field TCDD TEQs (WHO TEFs) in the range 410-25,400 pg/g (82 to 87% of TEQ derived from PCB 77) were linked to a high frequency of abnormal behaviour, and high rates of egg mortality in the Upper Hudson River, USA. For Eastern bluebirds, an LOAEL for embryo mortality of 10,000 pg TCDD/g fresh weight egg was derived in laboratory studies. An NOAEL for embryo mortality in the common goldeneye (*Bucephala clangula*) of 50,000 pg TEQ/g has been reported (Brünstrom and Reutergardh, 1986).

Based on the data cited above, and other information presented in Chapter 2, the suggested ranking of sensitivity of birds relative to chickens, from most sensitive to least sensitive, is as follows: chicken > wood duck > American kestrel, osprey, bald eagle > pheasant > double crested cormorant, blue heron, > turkey > mallard duck > goose > ring-billed gull > herring gulls > black-headed gull > Forster's tern, common tern > tree swallow, golden eye, eastern bluebird.

It is common practice in tier one risk assessments to use the most conservative endpoint for the most sensitive species in a first approximation of risk. As noted previously, the most conservative threshold TRV found in birds is an LOAEL of 9 pg TCDD TEQ/g wet weight egg for cardiac malformations in domestic chicken derived in laboratory studies by Cheung et al. (1981a & b, cited in US EPA, 2000d). This is equal to an NOAEL of 0.9 pg TEQ/g egg, assuming an extrapolation factor of 10 for deriving an NOAEL from an LOAEL.

While chickens are more sensitive relative to other tested birds, evidence does not suggest that domestication has made them inherently more sensitive to toxic chemicals than wild birds. This is because sensitivity is quite variable among all vertebrate species (e.g. fish, birds, and mammals). The gap between chickens and other birds is more likely to be a function of the relatively small number of species tested (Suter, 2003). Nevertheless, the

most sensitive reported endpoint for chickens is significantly lower than the mean NOAEL derived for chickens in other studies, and significantly lower than the TRVs provided in Table 4.2 for the other bird species. A TRV of 0.9 pg TEQ/g egg may therefore be overly conservative.

Consequently, for a first approximation of risk, the geometric mean of all available tests (n = 28) for embryo mortality and developmental effects in domestic chickens will be adopted to calculate a risk quotient. Using the geometric mean, the most sensitive species endpoint for the domestic chicken is 66 pg/g wet weigh egg. It is assumed that the mean NOAEL for the most sensitive bird species should be protective of other bird species in the environment. This TRV should also be protective of older birds because adult and juvenile birds are less sensitive than embryos.

Because most bird species collected in the survey are raptors, the most sensitive endpoint for the most similar species is also adopted as a more realistic TRV to derive a Q value. The most conservative TRV for a raptor species is the field NOAEL for bald eagle of 100 pg TEQ/g egg (wet weight). When dioxin residues in eggs fall below this threshold TRV, it suggests a low risk of developmental toxicity to wild raptor populations, recognizing also that there may have been additional confounding factors, such as other chemical and non-chemical stressors influencing effects based on the field measured exposures.

### 4.5.2 Exposure reference values

No data are available of egg exposure concentrations in Australian birds. However, dioxin body burden data are available for 19 individual birds, comprising 10 different species (Correll et al., 2004). These data show that dioxin levels in birds ranged from 1.0 pg TEQ/g lipid to 3506 pg TEQ/g lipid, using avian specific TEFs.

Bird embryos are exposed to dioxins through maternal transfer at the time of egg formation when dioxins present in the mother are transferred along with fat to the yolk of the egg. If we assume that the levels found in birds are representative of the maternal body burden in breeding female birds, the egg loads can be calculated from the maternal body burden, using the upper confidence limit of the transfer ratios (EMR) for PCDD/Fs and PCBs defined previously.

The bird body burden data are expressed in terms of TEQs on a lipid weight basis, while bird TRVs in the literature are reported on a whole egg wet weight basis. Thus, exposure concentrations in eggs must first be converted to whole egg wet weights. For this purpose, it is assumed that bird eggs contain 8% lipid (US EPA, 2003). The estimated TEQs in bird eggs are provided in Table 4.3. The resulting risk quotients are provided in Table 4.4.

**Table 4.3 Estimated TEQs in the bird eggs following maternal transfer**

| | | Maternal | Egg |
|---|---|---|---|
| **Species** | **Sampling location** | **pg/g lipid** | **pg/g egg ww** |
| Collared sparrowhawk | Adelaide, SA | 3506.2 | 245 |
| Sparrowhawk | Jane Brook, Perth | 1847.4 | 207 |
| Sparrowhawk | Beldon, Perth, WA | 995.2 | 72 |
| Black-shouldered kite | Elizabeth, SA | 805.6 | 85 |
| Black-shouldered kite | Penola, SA | 660 | 41 |
| Peregrine falcon | Adelaide, SA | 525.2 | 115 |
| Collared sparrowhawk | Penola, SA | 479.7 | 39 |
| Eagle-breast | Woodford, QLD | 259.4 | 22 |
| Peregrine falcon | Pt Lincoln, SA | 225.0 | 32 |
| Eagle-liver | Woodford, QLD | 204.5 | 20 |
| Kestrel female | Adelaide, SA | 192.2 | 127 |
| Hobby falcon, | Balga, Perth, WA | 122.4 | 8 |
| Kestrel male | Adelaide, SA | 117.7 | 9 |
| Brown goshawk | Penola, SA | 114.5 | 10 |
| Goshawk | Gympie, QLD | 90.7 | 10 |
| Pheasant coucal | Darwin, NT | 76.8 | 8 |
| Brown falcon | Darwin, NT | 50.7 | 5 |
| Black-shouldered kite | Bremer River, SA | 9.3 | 0.75 |
| Galah | Darwin, NT | 1.0 | 0.1 |

The predicted TEQs in raptor's eggs are within the same range as those reported in peregrine falcon (*Falco peregrinus*) eggs collected from the central coast of California in an area with no point source contamination. These eggs contained PCDD/Fs and PCBs at average TEQs of 125 pg/g wet weight whole egg, with PCB 126 comprising 83%. The levels in Californian falcon eggs were assumed to reflect bioaccumulation of background concentrations (Jarman et al., 1993). No soil levels or maternal body burden data were available for this study for comparison with the present study.

**Table 4.4 Risk quotients derived from predicted TEQs in bird eggs, and using the NOAEL for the most sensitive and most similar species**

| | | Risk quotient (Q) | |
|---|---|---|---|
| **Species** | **Sampling location** | **NOAEL chicken[1]** | **NOAEL bald eagle** |
| Collared sparrowhawk | Adelaide, SA | 3.7 | 2.45 |
| Sparrowhawk | Jane Brook, Perth | 3.1 | 2.07 |
| Kestrel female | Adelaide, SA | 1.9 | 1.27 |
| Peregrine falcon | Adelaide, SA | 1.7 | 1.15 |
| Black-shouldered kite | Elizabeth, SA | 1.3 | 0.85 |
| Sparrowhawk | Beldon, Perth, WA | 1.1 | 0.72 |
| Collared sparrowhawk | Penola, SA | 0.6 | 0.39 |
| Black-shouldered kite | Penola, SA | 0.6 | 0.41 |
| Peregrine falcon | Pt Lincoln, SA | 0.5 | 0.32 |
| Eagle-breast | Woodford, QLD | 0.3 | 0.22 |
| Eagle-liver | Woodford, QLD | 0.3 | 0.20 |
| Goshawk | Gympie, QLD | 0.2 | 0.10 |
| Brown goshawk | Penola, SA | 0.2 | 0.10 |
| Kestrel male | Adelaide, SA | 0.14 | 0.09 |
| Hobby falcon, | Balga, Perth, WA | 0.12 | 0.08 |
| Pheasant coucal | Darwin, NT | 0.12 | 0.08 |
| Brown falcon | Darwin, NT | 0.07 | 0.05 |
| Black-shouldered kite | Bremer River, SA | 0.01 | 0.0075 |
| Galah | Darwin, NT | 0.001 | 0.001 |

[1]based on the geometric mean of 28 studies

The risk quotients (Q values) shown in Table 4.4 indicate that 6 out of 19 birds have a Q value greater than one, when the TRV for the most sensitive species (domestic chicken) is applied. Using the TRV for the most similar species, 4 out of 19 birds have Q values near to or greater than one.

In the literature, a number of more conservative TRVs have been suggested and applied to assess the risk of dioxins to birds (Table 4.5). For example, Koistenen et al., (1997) suggested a threshold concentration of 7 pg/g egg (pg/g) to protect Baltic white-tailed sea eagles from adverse effects of dioxins. These authors based the threshold on the $LC_{50}$ for the American kestrel exposed to PCB 126 because no data are available for TCDD toxicity to sea eagles. The threshold was calculated using the potency factor of PCB 126 relative to TCDD (0.015 for avian species), and assuming a factor of 100 for extrapolation from the

$LC_{50}$ to LOAEC, based on the ratio for TCDD toxicity in white leghorn chickens (ASTM, 1993). Using this TRV, more than 80% of eggs have Q value above one.

**Table 4.5 TRVs used in risk assessments of avian wildlife**

| Response | Level | Effect | Reference |
|---|---|---|---|
| Based on hepatic induction in White-tailed sea eagle | 1500 pg/g ww liver<br>300 pg/g egg | NOAEL | Kannan et al. (2003) |
| Based on the LC50 for PCB 126 and relative toxicity ratio of PCB 126 to TCDD in the American kestrel (EF factor of 100) | 105 pg/g ww liver<br>7 pg/g ww egg | LOAEL | Koistenen et al. (1997) |
| Based on hepatic CYPIA induction as biomarker in Bald eagle | 100 pg/g egg | NOAEL | Elliott et al. (1996) |
| Based on developmental abnormalities, such as beak deformities, in Double-crested cormorants | 20 pg/g egg | LOAEL | Powell et al. (1998) |
| Based on toxicity of PCBs to the American kestrel (UF = 3.5) | 70 pg/g egg | LOAEL | Kemler et al. (2000) |

In another assessment, Kemler et al., (2000) derived a TRV for the protection of the American kestrel of 70 pg TCDD/g egg, based on the NOAEL for PCB 126 of 2300 pg/g wet weight egg, and applying a TEF of 0.1 and an uncertainty factor of 3.5. Using the Kemler TRV, more than 30% of eggs exceed Q = 1.

Uncertainty factors (UF) are often applied to TRVs when the endpoint in some way does not represent the species or toxicant of interest. In the Kemler study, a UF of 3.5 was applied because of laboratory to field extrapolation and because of uncertainty in the NOAEL endpoint for the toxicant. Application of a higher UF could also be argued when the species for which the toxicity endpoint was derived differs from the species it is being applied to.

### 4.5.3 Species sensitivity distributions

In a recent document, Suter (2003) proposed the use of species sensitivity distribution (SSD) curves to assist ecological risk assessors to characterise the risk to birds from exposure to dioxin-like chemicals. The SSD endpoints proposed by Suter (2003) are derived from the NOAEL for embryo mortality and developmental impairment in birds determined in laboratory studies for 10 different bird species. The approach uses the geometric mean of all available effect concentrations for a given species, rather than just the most sensitive endpoint and species (Table 4.6).

Figure 4.2 shows the calculated TEQs in Australian bird eggs (wet weight) (data from Table 4.4) compared to the SSD of the geometric mean NOAEL based on the data from Table 4.6.

**Table 4.6 Geometric mean NOAELs for embryo mortality and developmental impairment in birds exposed to dioxins in laboratory studies**

| Species | Scientific name | NOAEL pg/g | Number of tests |
|---|---|---|---|
| White leghorn chicken | *Gallus domesticus* | 66 | 28 |
| American kestrel | *Falco sparverius* | 230 | 1 |
| Ring-necked pheasant | *Phasianus colchicus* | 710 | 2 |
| Double-crested cormorant | *Phalacrocorax auritus* | 3670 | 4 |
| Turkey | *Meleagris gallopave* | 10000 | 1 |
| Mallard duck | *Anas platyrhynchos* | 35360 | 2 |
| Greylag goose | *Anser anser* | 50000 | 1 |
| Common goldeneye | *Bucephala clangula* | 50000 | 1 |
| Black-headed gull | *Larus ridibundus* | 50000 | 1 |
| Herring gull | *Larus argentatus* | 50000 | 1 |

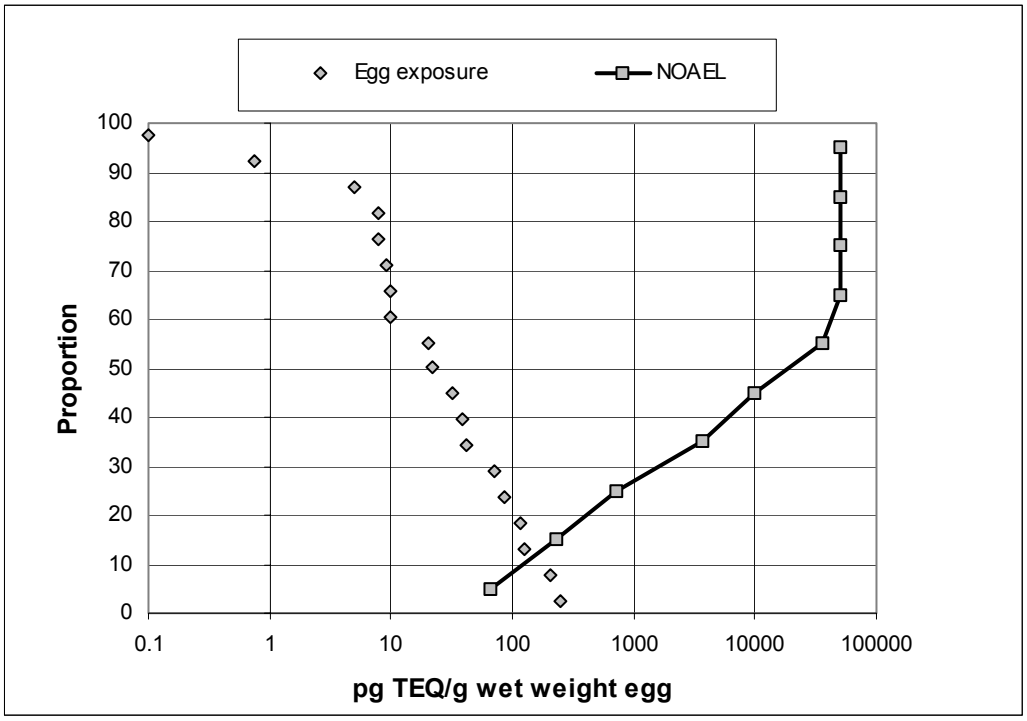



**Figure 4.2 Species sensitivity distribution and TEQs in bird eggs**

The results indicate that approximately 30% of egg exposure concentrations are higher than the NOAEL for domestic chicken, while less than 10% of eggs are above the threshold for the most similar species (American kestrel). These results suggest a cause for concern and suggest that the levels found in some of the individual raptors, may be high enough to cause

adverse effects in young birds, at least in those bird populations residing in the areas where the samples with the highest dioxin loads were found. For the most part, these birds were collected in urban environments. However, the results are based on a relatively small number of TRVs derived from laboratory studies and a small data set of deceased birds collected opportunistically and therefore caution is required in generalising these results to entire populations of birds in the natural environment.

In studies with wild populations of birds in Wisconsin, USA, TCDD concentrations in osprey eggs between 29 and 162 pg/g ww were associated with a decrease in chick growth rates but not hatching success (Woodford et al., 1998). TEQs, calculated on a whole egg basis ($WHO_{98}$ avian TEFs) between 77 and 126 pg/g also did not impact hatching success of wild osprey from British Columbia, however, biochemical parameters including increased hepatic EROD activity, and higher tissue concentrations of some vitamin A compounds were positively correlated with TEQs. No correlation was found for morphological and histological parameters such as edema, deformities, and renal and hepatic porphyrin levels (Elliott et al., 2001).

Maximum dioxins levels of 258 pg TEQ/g (based on the TEF system of Safe, 1990) in osprey eggs (whole egg, wet weight) at contaminated sites downstream of a bleached kraft paper mill on the Wisconsin River did not significantly impact egg hatching and chick fledging rates, but resulted in a lower mass increase rate in chick growth than in chicks at reference sites downstream of the mill, having lower dioxin levels (maximum 115 and 96, PCDD/Fs and PCBs combined) (Woodford et al., 1998).

These studies with wild bird populations suggest that, at the levels found in Australian raptors, breeding in terms of hatching success and fledging rates, may not be impacted, however, the overall health of birds, in terms of biochemical parameters such as vitamin A metabolism and chick growth rates could be compromised.

In summary, the results of the avian risk assessment signal a potential cause for concern for high trophic level raptors. When the TRVs are expressed as species sensitivity distribution curves, using all available laboratory data, approximately 30% of egg exposure concentrations are higher than the geometric mean NOAEL (n = 28 endpoints) for the most sensitive species, the domestic chicken, while approximately 20% of egg exposure concentrations are above the threshold for the bald eagle, which would induce hepatic CYP1A, and about 10% of eggs are above the threshold for embryo mortality in the American kestrel.

### 4.5.4 Exposure to bird embryos based on soil concentrations

To further characterise risk to birds, additional egg TEQs are predicted from the measured dioxin levels in soil and using the average BSAFs for raptors. For this purpose, the bird BSAFs are divided into two groups, agricultural/remote and urban/industrial, according to the land-use categories from where the birds were collected. The proportion of eggs that exceed a given toxicity threshold for a given soil level (n = 104) are shown in Figure 4.3, together with the SSD of the NOAEL for embryo mortality determined for different bird

species in laboratory studies (based on data from Suter, 2003). The egg TEQs determined from the measured tissue concentrations are included for comparison.

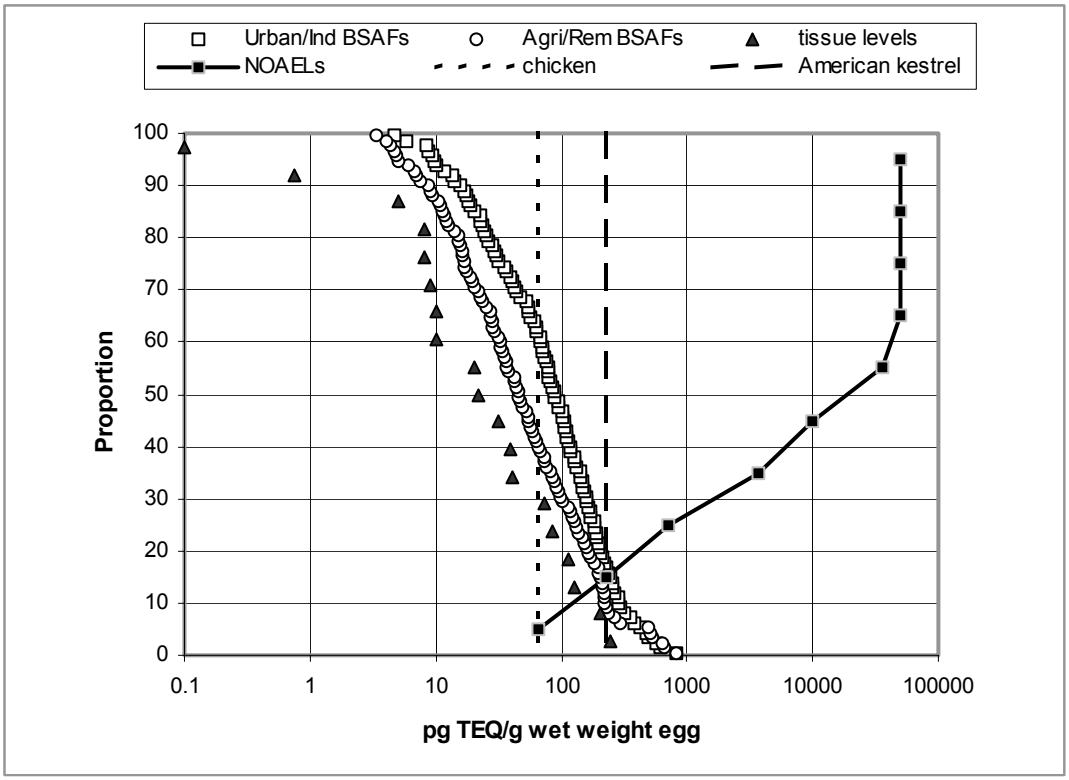



**Figure 4.3 Species sensitivity distribution and TEQs in raptor eggs calculated from tissue levels and predicted from BSAFs**

The predicted TEQs in eggs, based on soil concentrations, varied markedly among individual birds depending on the BSAF and soil levels. For birds in agricultural areas, the predicted mean and median TEQ in eggs were 102 pg/g and 46 pg/g, wet weight (range 3 to 825 pg/g). For birds from urban/industrial areas, the predicted mean and median TEQ in eggs were 133 pg/g and 95 pg/g, wet weight (range 3-803 pg/g). These predicted values are on average about two to three times higher than those derived from the measured tissue concentrations, for which the mean and median TEQs in eggs were 62 pg/g and 32 pg/g, wet weight (range 5 to 245), respectively.

Figure 4.3 shows that the threshold egg concentrations, based on the NOAEL for the American kestrel (230 pg/g egg), are on average exceeded (exceedence points are located where the exposure distribution curves cross the vertical effects distribution curves) in eggs at about 10 to 20% of soil concentrations, using ambient soil levels and the average BSAFs for both urban/industrial and agricultural/remote birds, compared to about 10% for those calculated from measured data. The threshold egg concentrations, based on the NOAEL for the domestic chicken (i.e. 66 pg/g), are exceeded at about 60% of ambient soil exposure concentrations, using the average BSAFs for urban/industrial birds, about 40% of ambient

soil exposure concentrations using the average BSAFs for agricultural and remote birds. This compares to about 30% exceedence for TEQs calculated from the measured data.

There are many reasons why the BSAFs may not accurately predict tissue levels. These reasons are discussed below and elsewhere in the report and will not be repeated here. Neverthless, because of biomagnification, birds high in the food chain do appear to accumulate relatively high levels of dioxins from background exposure levels compared to many other wildlife species. For example, relative to fish, mammals, and krill, Antartic birds were found to accumulate the highest levels of dioxins, with birds that predate on other birds accumulating the highest levels. Levels found in Antarctic wildlife were in the following increasing order: krill 0.58 pg TEQ/g lipid, fishes 0.6 to 2.4 pg TEQ/g lipid, Weddell seal liver 23 pg TEQ/g lipid, penguin eggs 28 pg TEQ/g lipid (5.6 pg/g wet weight), skua eggs 220 to 650 pg TEQ/g (32 pg TEQ/g wet weight). The higher TEQ is skua eggs compared to penguin probably reflect dietary differences. South polar skua predate on other birds, while Adelie penguin feed almost exclusively on fish and krill (Kumar et al., 2002).

By world standards, dioxin levels are comparitively low in Australian terrestrial birds (refer to Corell et al., 2004 for data). Dioxin levels in wildlife in the southern hemisphere are also lower than in wildlife in the northern hemisphere, reflecting the greater number of industrial emission sources for PCDD/Fs and PCBs in the northern hemisphere compared to the southern hemisphere. For example, Tanabe *et al* (2004) found TEQs (based on $WHO_{98}$ TEFs, and including PCDD/Fs and dioxin-like coplanar PCBs) in albatross from the remote Southern Ocean, west of the Australian continent, were much lower than those in albatross from the remote North Pacific Ocean. Levels reported for the Southern Ocean birds were between 62 pg/g lipid and 120 pg/g lipid, depending on species, while for the North Pacific the levels were between 3600 pg/g lipid and 6000 pg/g lipid.

TEQs in albatross from the remote Southern Ocean are generally lower than the TEQs in most Australian terrestrial raptors (Table 4.3), while for the North Pacific, levels in albatross were higher than those found in many terrestrial birds collected from contaminated areas, such as the Great Lakes of North America, but also lower than those reported for many terrestrial raptor species from a number of different locations worldwide (Kumar et al., 2002; Tanabe et al., 2004). These data also support the findings that raptors accumulate higher concentrations of dioxins compared to other bird species.

The TEQs in Australian raptors are about the same order of magnitude as those reported in terrestrial raptors in India, while being generally lower than the TEQs in raptors from more northern regions. For example, a TEQ of 240 pg/g lipid was reported in the muscle of a praire kite, and a TEQ of 150 pg/g lipid was found in the muscle of a spotted owlet from India (Tanabe et al., 2004). A TEQ of 5800 pg/g was found in the liver of a goshawk, and a TEQ of 4300 pg/g was found in the liver of a black-eared kite, both from Japan, while a TEQ of 16000 pg/g lipid was found in the liver of white-tailed sea eagle in Germany. In general, TEQs in bird eggs are approximately 15 times lower than TEQs in the liver (Kannan *et al* 2003).

Tanabe et al., (2004) found that among the PCDD/F congeners, 2,3,4,7,8-PeCDF was the predominant congener in albatross. This is also a common pattern in fish-eating birds. While this congener was not the dominant one in Australian birds (OCDD was), 2,3,4,7,8-PeCDF consistently had the highest BSAF, suggesting that it preferentially accumulates in birds. OCDD was the dominant PCDD/F congener in Antarctic seal and krill, while TCDF or 2,3,4,7,8-PeCDF were dominant in fish, penguin and skua eggs (Kumar et al., 2002). OCDD and 1,2,3,7,8-HCDD were generally the dominant PCDD congeners in albatross from the Southern Ocean, while OCDD levels were relatively low in birds from the North Pacific Ocean. The higher OCDD levels in the Southern Ocean albatross probably reflect the predominance of this congener in soil and sediment in Australia, rather that its bioaccumulation potential. The BSAF for OCDD in raptors was consistently lower than other PCDD/F congeners, indicating its lower bioaccumulation potential.

For PCBs, Tanabe et al., (2004) found the dominant congeners in albatross were PCB 118> PCB 105 > PCB 156 > PCB 167. For Australian raptors, the same congeners were dominant, although the relative order differed, as follows: PCB 118 > PCB 167> PCB 156 > PCB 105. In Antarctic wildlife, PCBs contributed about 75% of TEQs, with PCB 105 and PCB 118, the dominant mono-*ortho* PCBs in penguin and skua eggs (Kumar et al., 2002).

## 4.6 Risk to mammals

### 4.6.1 Aquatic mammals

There are very few toxicity benchmarks available for aquatic mammals exposed to dioxins. The best available threshold estimates for use in screening level risk assessment are presented in Kannan et al., (2000). These authors suggest lipid normalised threshold concentrations of between 160 and 1400 pg TEQ/g lipid for the protection of aquatic mammals. The threshold TRVs are derived from published laboratory, semi-field and field studies of PCB toxicity in seals, mink and otter.

Threshold PCB concentrations in the liver of seals, mink and otter that elicit physiological effects (including immune suppression and reproductive effects) range between 6.6 and 11 µg PCBs/g (geometric mean: 8.7 µg/g). In seals and dolphins, lipid normalised concentrations of PCBs in the blubber are usually two-fold greater than in the liver and blood, while in marine mammals in general, they are within a factor of two to five. Based on the relationship between blubber and liver PCB concentrations, Kannan et al., (2000) determined a threshold value of 17 µg (17,000 ng) PCBs/g lipid, which is equivalent to about 160 and 1400 pg TEQ/g lipid (based on $WHO_{1994}$ TEFs) (geometric mean of 3 values: 520 pg TEQ).

Using the geometric mean (520 pg TEQ/g lipid) and the lowest TRVs (160 pg TEQ/g lipid), the resulting Q values calculated for aquatic mammals found in the Australian marine environment are provided on Table 4.7. The table also provides total PCBs concentrations, excluding the LOD, found in marine mammals (Correll et al., 2004).

The bottlenose dolphin from Port Adelaide exceeds the threshold value, assuming the mean TRV, and both dolphins exceed the threshold, assuming the lowest TRV. This suggests that these animals could experience adverse effects from exposure to dioxins. The other individuals are below the threshold values likely to cause adverse effects.

**Table 4.7 PCBs (ng/g lipid), TEQs and resulting risk quotients in aquatic mammals[19]**

| Species | Sampling location | PCB ng/g lipid* | TEQ pg/g lipid | $Q_{mean}$ | $Q_{lower}$ |
|---|---|---|---|---|---|
| Bottlenose dolphin | Port Adelaide, SA | 2800 | 585 | 1.1 | 3.7 |
| Humpback dolphin | Darwin, NT | 1000 | 190 | 0.36 | 1.2 |
| Sperm Whale | Waterhouse, TAS | 38 | 42 | 0.08 | 0.3 |
| Sperm Whale | Waterhouse, TAS | 37 | 39 | 0.08 | 0.2 |
| Sperm whale | West Coast, TAS | 34 | 37 | 0.07 | 0.2 |
| Sperm Whale | Waterhouse, TAS | 24 | 31 | 0.06 | 0.2 |
| Sperm Whale | Waterhouse, TAS | 26 | 29 | 0.06 | 0.2 |
| Beaked whale | Cloudy Bay, TAS | 18 | 27 | 0.05 | 0.2 |
| Sperm Whale | Waterhouse, TAS | 50 | 24 | 0.05 | 0.15 |
| Australian sea lion | Seal Bay, SA | 63 | 23 | 0.04 | 0.1 |
| Long fin pilot whale | Sisters Beach, TAS | 17 | 21 | 0.04 | 0.1 |
| Sperm Whale | Waterhouse, TAS | 76 | 20 | 0.04 | 0.1 |
| Dugong | Darwin, NT | 0.18 | 2 | 0.004 | 0.01 |

*excludes LOD

In a study by Ruchel (2001), dioxin body burdens were determined for two dolphins from Spencer Gulf in QLD and for two dolphins from Port Adelaide, South Australia. TEQs in the Spencer Gulf dolphins were 12.05 and 39.3 pg TEQ/g lipid (half LOD), which is significantly lower than the TRV range, while TEQs in the dolphins from Port Adelaide were 290 and 450 pg/g lipid, which falls within the threshold range. The TEQs in the Adelaide dolphins are about the same order of magnitude as those found in the dolphin samples collected in the NDP survey (Corell et al., 2004).

The measured TEQs in dolphins are within the same order of magnitude as those reported in a review paper by Colborn and Smolen (1996), for harbour porpoises from the Baltic Sea, while being lower than the TEQs reported in striped dolphins and whales from various other locations. For example, 9 of the 16 cetacean populations for which samples are available had body burden TEQs of 500 pg/g, harbour porpoises were carrying 526 pg TEQ/g lipid, and striped dolphins were carrying 6,676 pg TEQ/g lipid, at the time of sampling (based on the TEFs of Safe, 1990). These TEQs are, however, based on a small number of dioxin-like PCBs and, therefore, represent conservative estimates.

Studies from around the globe indicate that PCBs are the major contributor to dioxin-like toxicity in marine mammals (Kumar et al., 2002). This is particularly true for open ocean dolphins, which are found to accumulate higher PCB burden than coastal and terrestrial mammals (Tanabe et al., 2004). For the Australian aquatic mammals, PCBs also

[19] Q values are based on the lower threshold and mean threshold TRVs

contributed the highest TEQ, except for the herbivorous dugong. In the two dolphins and the sea lion, PCBs contributed 90 to 99% of the TEQ, while in the whales, PCBs contributed over 80% of TEQ.

The minimum or lower range concentrations of PCBs found in the livers of diseased or dead marine mammals, including seals, porpoises, sea otters, and dolphins, from coastal waters in the United Kingdom, the United States, and Northern Europe, are in the range of 0.06 to 7 µg/g (60 to 7000 ng/g) PCBs on a wet weight basis (Kannan et al., 2000). The PCB levels found in the lipid of Australian aquatic mammals are lower, in the range 17 to 2800 ng/g lipid PCBs (i.e. maximum 2.8 µg/g, excluding the dugong) (Table 4.7). If we assume 43% lipid (a conservative estimate, McLellan et al., 2002), these levels equate to wet weight body burdens between 8 and 1204 ng/g, with four mammals being above the lower range value of 60 ng/g. The PCB body burdens found in the dolphins are therefore in the lower end of the range suspected to cause disease and mass mortalities observed in other regions. However, if we allow for a five fold higher concentration in blubber relative to liver, these values would fall within the mid to high end of the range.

As noted previously, linking chemical residue levels in marine mammals in the natural environment with disease and mass mortality events is difficult owing to the large number of stressors, other that chemicals, that could cause these effects. Other stressors linked to disease and death in marine mammals, include exposure to natural marine toxins, and bacterial, viral, and parasitic infections (EPA, 2002).

In summary, the TEQ levels found in dolphins, combined with the TRVs derived from laboratory and semi-field studies with aquatic mammals, suggest that the health of dolphins living in the vicinity of urban/industrial estuaries may be at risk from exposure to dioxins. At the highest levels observed in dolphins, i.e. 190 pg TEQ/g lipid (185 pg using ½ LOD) and 585 pg (both full and ½ LOD), the presence of subtle adverse effects on the immune system and reproduction cannot be ruled out, based on the lower threshold TEQ values for these effects found in overseas studies.

### 4.6.2 Predicted exposure based on sediment concentrations

Figure 4.4 shows the predicted TEQs in aquatic mammals (lipid weight basis) calculated from the BSAFs for individual mammals and the dioxins levels found in marine and estuarine sediments. The lower and upper TRVs suggested by Kannan *et al* (2000) for risk assessment are also shown. The BSAFs were calculated from the average dioxin levels found in estuarine and marine sediments (n = 39) for all locations combined. This approach takes into account the large home ranges and varying exposure concentrations encountered by aquatic mammals. The resulting body burdens are assumed to represent the maximum level that could be achieved at a given sediment concentration, assuming the BSAF applies to that location and the organism is exposed only to that concentration. However, the estimates should be treated cautiously owing to uncertainties in the BSAF.

The figure indicates that 10 to 25% of dolphins have body burdens above the maximum TRV expected to cause adverse effects. All of the data points resulting in TRVs exceeding the threshold are located in urban estuarine environments in Melbourne, Sydney and

Brisbane. This would suggest that dolphins eating fish exposed exclusively to sediment concentrations at the levels found in these estuaries and ports could theoretically achieve body burdens significantly higher than the threshold TRVs. In reality, this scenario is highly unlikely, since dolphins are likely to spend a large portion of their time feeding in the open ocean.

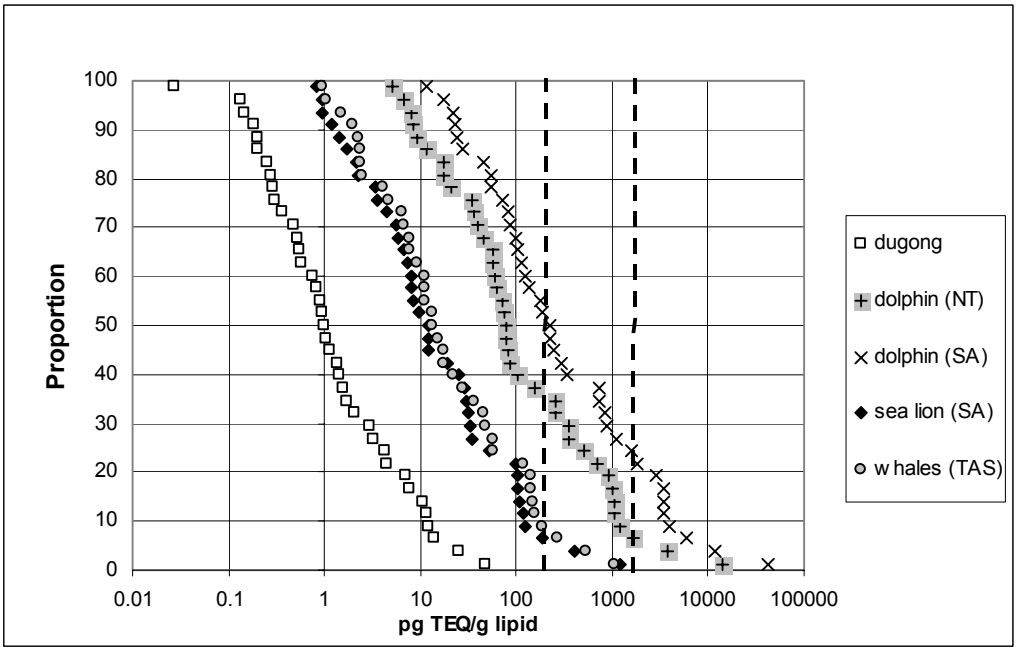



**Figure 4.4 Predicted TEQs in aquatic mammals, using average BSAFs, compared to the range of threshold TRVs (vertical dashed lines)**

### 4.6.2 Terrestrial mammals

A large database is available examining the toxicity of TCDD to a limited number of terrestrial laboratory mammals. The majority of these experiments are performed with rats and mice exposed to dioxins in their food, making these TRVs incompatible with the exposure concentrations based on body burden collected during the NDP survey.

For placental mammals, dioxins are thought to disrupt the development of the endocrine, reproductive, immune and nervous system at critical stages during gestation at relatively low concentrations. As such, threshold maternal body burdens at steady state have been determined by the EU (2001) from which to develop corresponding estimated human daily intakes to protect human babies from developmental effects when exposed via maternal transfer during gestation. The EU committee was not able to identify any single study as being sufficient by itself to derive threshold values. Instead, it based the threshold on a group of LOAELs for sensitive developmental effects in young male rats when the pregnant females were exposed to 2,3,7,8-TCDD in their food during critical phases of gestation (generally gestation day 15). The final threshold body burden values are derived from estimated and measured maternal and foetal 2,3,7,8-TCDD body burden (Table 4.8).

Studies reviewed in the EU document indicate that the ratio (i.e. maternal/foetal) between the maternal body burden and the foetal body burden (based on pg/g bw) range between 5.7 and 13.4 for a single dose exposure and between 14.3 and 20 for subchronic exposure, depending on dose. The ratio generally increases with increased dose. Thus, at the lowest subchronic dose, the foetal body burden is about 7% of the maternal body burden, while at the highest subchronic dose, the feotal body burden is about 5% of the maternal body burden.

**Table 4.8 NOAELs and LOAELs for developmental impairment in rats following exposure to TCDD**

| Species | Endpoint | Maternal body burden[1] | Dose/effect level |
|---|---|---|---|
| Holzman rats | Decreased sperm count in male offspring | 100 pg/g bw[2]<br>833 pg/g lipid | LOAEL: 64 pg/g bw single bolus dose by gavage |
| Long Evans rats | Accelerated eye opening and decreased sperm count in male offspring | 80 pg/g bw[2]<br>667 pg/g lipid | LOAEL: 50 pg/g bw single bolus dose by gavage |
| Wistar rats | Production and altered sexual behavior in male offspring | 40 pg/g bw[2]<br>333 pg/g lipid | Maintenance of 25 pg/g bw by subcutaneous injections |
| Holzman rats | Decreased anogenital distance in male offspring | 80 pg/g bw[3]<br>667 pg/g lipid<br>20 pg/g bw[3]<br>167 pg/g lipid | LOAEL: 50 pg/g bw single bolus dose by gavage<br>NOAEL: 12.5 pg/g bw single bolus dose by gavage |

[1]Increment over background. Background body burden in rats is about 4 ng TEQ/kg bw.
[2]Composite value resulting from pseudo steady state body burden and acute body burden on GD 15.
[3]Maternal body burden at GD 16.

Toxicity threshold values have also been developed for mink, which are one of the few wildlife species studied in laboratory experiments for their sensitivity to TCDD. Studies with mink show them to be among the most sensitive mammalian species to the toxic effects of dioxins. Toxicity data for mink are summarised in Table 4.9. The mink TRVs are based on wet weight dioxin levels in the liver.

If we assume that the lipid content of mink liver is 5% (Kannan et al., 2000), and the liver NOAEL is equivalent to the whole body NOAEL, the TRV for reproductive effects in mink is 220 pg TEQ/g lipid. Similarly, if we assume that test rats used in the EU document have a lipid content of 12% of body weight, the resulting threshold values expressed on a lipid weight basis, are between 167 pg TEQ/g lipid for the NOAEL and 500 pg TEQ/g lipid for the LOAEL, respectively. These threshold values are within the same range as the threshold TRVs derived for aquatic mammals.

To characterise the risk to terrestrial wildlife, the lowest TRV of 167 pg/g lipid (based on the maternal body burden) will be adopted to derive the risk quotients for Australian terrestrial fauna. This approach assumes that the ratio of transfer from mothers to

developing young is similar for marsupials. It also assumes that marsupials have a similar sensitivity to dioxins in terms of reproductive toxicity as placental mammals. Furthermore, it does not assume any safety factors commonly adopted to deal with inter species uncertainty. The results are provided in Table 4.10.

The highest dioxin level found in a terrestrial mammal was in an echidna from Port Elliot, SA, and the next highest level was found in a Western grey kangaroo from Para Wirra, SA. The resulting Q values for these animals, and other species of terrestrial mammals showing the highest levels for their group, are all less than one, assuming a TRV of 167 pg/g lipid. These results indicate that body burdens found in Australian native mammals are below the toxicity threshold derived for placental mammals (Table 4.9).

**Table 4.9 A summary of toxicity endpoints in mink based on tissue concentrations**

| Effect level | Exposure | Response | Reference |
|---|---|---|---|
| EC50 | 1.2 µg/g PCBs/ wet weight muscle<br>40-60 µg/g PCBs/lipid weight (2-3% lipid)<br>0.16 ng/g TCDD equivalence/ww | Litter size | Leonards et al., (1995) |
| EC50 | 2.4 µg/g PCBs/ww muscle<br>0.20 ng/g TCDD equivalence/ww | Kit survival | Leonards et al., (1995) |
| NOAEL | 11 pg TEQ/g liver ww<br><10 pg H4IIE TCDD-EQ/g liver weight | Reproductive effects | Tillitt et al., (1996) |
| LOAEL | 324 pg TEQ/g liver weight<br>495 pg H4IIE TCDD-EQ/g liver weight | Reproductive effects | Tillitt et al., (1996) |
| Threshold | 60 pg TEQ/g liver weight<br>70 H4IIE TCDD-EQ/g liver weight | Reproductive effects | Tillitt et al., (1996) |

**Table 4.10 TEQs and Q values in terrestrial mammals, using a TRV of 167 pg/g lipid**

| Species | Sampling location | pg TEQ/g lipid | Q |
|---|---|---|---|
| Echidna | Port Elliot, SA | 60 | 0.34 |
| Western grey kangaroo | Para Wirra, SA | 25.4 | 0.15 |
| Brush tail possum | Hanson Bay, SA | 13.5 | 0.08 |
| Platypus | Glengarry, TAS | 9.7 | 0.06 |
| Dingo | Ceduna, SA | 2.89 | 0.02 |
| Northern Brown bandicoot | Jabiru, NT | 2.1 | 0.01 |
| Koala | Crafers, SA | 1.45 | 0.009 |

The results should be viewed as a screening guide only owing to the uncertainty associated with choosing appropriate threshold TRVs for Australian native mammals. There is essentially no information regarding the toxicity of dioxin in marsupials, nor of their ability

to metabolise and eliminate these chemicals. The variability in sensitivity among the different marsupial and monotreme species, and between marsupial and placental mammals, is also not known.

As a result of their reproductive strategy, it has been suggested that marsupials are potentially more vulnerable to chemical exposure during their development in comparison to placental mammals (Bolton and Ahokas, 1995). The TRV for terrestrial mammals is based on exposure by placental transfer at gestation day 15 or 16. In mammals, the transfer of dioxins from mother to offspring during lactation is also an important route of exposure to newborns, with placental transfer of dioxins generally being much lower than in milk, particularly for mammals with lipid-rich milk.

The major differences in the reproductive strategy between marsupials and placental mammals are that placental contact is comparatively short for marsupials. Undeveloped neonates emerge after a short gestation period (up to a month) and relocate to the maternal pouch where they continue to develop, obtaining nourishment exclusively by lactation. The reproductive strategy of the monotremes (platypus and echidna) add an additional level of complexity to determining exposure, in that monotremes first produce one or two eggs, and after the eggs are laid, they are incubated externally from the mother for between six and ten days. At the end of incubation the young break free and then feed on milk from the mother for around three to four months until they become independent (Grant, 1989). It is thought the gestation period for egg development in the mother is similar in both platypus and echidna, and takes about one month.

While the TRVs derived from exposure at GD 15 and 16 should cover the gestation period for most marsupials and monotremes, the marsupial and monotreme neonates would be prone to continued exposure to dioxins during lactation, at a time when physiologically development is still incomplete. The ramifications of these differences for selecting TRVs based on embryo exposure at critical stages of development are not clear, and these information gaps add additional uncertainties to the risk determination for terrestrial mammals.

The amount of dioxin transferred via maternal milk varies, depending on the fat content of the milk and the duration of lactation. The fat content of marsupial milk changes over time to meet the nutritional requirements of the developing young. The changes vary between species, but the fat content is generally about 1.5% in the early phases of lactation, and increases to about 10% in the latter phases of milk production. Thus, compared to dolphins, porpoises, and whales, who produce the most lipid-rich milk (30 to 50%) of all mammals, the fat content in marsupial milk is low. This low fat content in early phase milk could be expected to minimise dioxin transfer in early life stage marsupials, at least compared to aquatic mammals.

## 4.7 Uncertainty analysis

All risk assessments involve uncertainty, which can influence the estimation of risk, as they are a prediction of the likely impact and the likelihood of a particular situation. Sources of uncertainty are introduced at all stages in the risk assessment including in the development

of the conceptual models, in the exposure and effects assessments, and in the risk determination. Some uncertainties will lead to an overestimation of the risk, while others may lead to an underestimation of the risk. In the following sections, the main sources of uncertainty in the risk assessment are described. It is also important to evaluate the level of confidence that can be ascribed to the risk estimation as a result of these uncertainties. Thus, an attempt is also made to determine whether the uncertainties are likely to over or underestimate the risk, although, no attempt is made to quantify the uncertainty.

### 4.7.1 Uncertainties in the conceptual model

A source of uncertainty associated with the conceptual model used in this ERA is the adoption of the "toxicity equivalence" methodology. The validity of the "toxicity equivalence" method depends on two important assumptions. The first is that the individual dioxin-like congeners act via the same mechanism to cause toxicity, and the second is that these toxic effects are additive. This methodology therefore does not take into account PCDDs, PCDFs, and PCBs, which may be present in a mixture that do not have toxic effects mediated by the Ah receptor, but may be more toxic through other mechanisms of action. It also does not take into account synergistic toxicity. These factors might result in an underestimation of risk when non-dioxin like congeners are present, but are not included in the conceptual model.

The best and most current TEF system is based on the potency of dioxin-like chemicals relative to TCDD (Van den Berg et al., 1998) and considers all available data. The uncertainties associated with this system are outlined in Van den Berg et al., (1998) and will not be repeated here. It is sufficient to say that, according to the US EPA (2003), the uncertainties associated with using the TEF methodology are not thought to be larger than other sources of uncertainty within the ecological risk assessment process. The TEF systems are derived to be conservative and hence are likely to overestimate the toxicity of some congeners.

Uncertainties in the TEF system can be reduced by selective relative potencies that best reflect the TRVs and species of concern to which TRVs are applied. The use of TCDD TEQ based on $WHO_{98}$ fish TEFs is expected to provide a good indication of the toxicity of the dioxin mixtures to fish as these TEFs are derived from relative potency data for mortality of early life stage rainbow trout exposed as embryos, although some data are also derived from *in vitro* studies for cytochrome (CYP1A) induction. Similarly, bird-specific TEFs are expected to provide a good indication of the toxicity of the dioxin mixtures to bird embryos, as the TEFs are derived from egg injection studies, and *in vitro* studies (i.e. with cultured avian hepatocytes and thymus cells) for a number of different bird species. Mammalian TEFs, by comparison, are based largely on rodent studies exposed to dioxins in their diet, and it is unclear whether these provide the best indication of relative potency of dioxins for wildlife, particularly native Australian wildlife, when TRV and exposure is derived in terms of body burden.

### 4.7.2 Uncertainties associated with effects

The greatest source of uncertainty associated with the effects assessment is the lack of toxicity data for Australian wildlife species. Toxicity information on developmental effects in early life-stages is limited to a few species of (mainly northern hemisphere) birds, fish and mammals. The relative sensitivity of Australian native species compared to the most sensitive test species is not known, and hence, represents a major uncertainty in any risk assessment.

Extrapolating from laboratory endpoints derived from different exposure conditions, duration, and routes (food, injection) to the natural environment are also major sources of uncertainty, although the use of body burden as measures of exposure and response reduces some of the uncertainty associated with this. Extrapolating laboratory toxicity data to toxicity in the natural environment, can overestimate risk when it does not take into account the ecological factors occurring under natural conditions that can help to limit exposure and the adverse effects (e.g. bioavailability, avoidance, adaptation). Alternately, laboratory tests may miss some critical subtle effect that can have significant impacts on what happens in the natural environment. The selection of the most sensitive species and endpoint is intended as a conservative approach to prevent underestimation of risk, but is not foolproof owing to the lack of information on the toxicity of dioxins to Australian native wildlife. The lack of toxicokinetic information (rates of uptake, distribution and elimination) in wildlife, particularly Australian wildlife, makes it difficult to even make an educated guess of comparative sensitivity using a weight of evidence approach.

Toxicity reference values derived from field studies (e.g. birds and aquatic mammals) are associated with uncertainties arising from the many confounding factors in the natural environment, such that cause-effect linkages between disease development, mortalities, and chemical contamination cannot be easily established.

### 4.7.3 Uncertainties associated with exposure

The main uncertainties associated with the exposure assessment are associated with the sampling strategy, the small number of samples, and the high variability among samples available from the NDP survey. Opportunistic sampling of species results in uncertainties associated with a lack of replication, sampling bias, failure to measure random variation, lack of species and food chain representation in the exposure assessment. As a result of these uncertainties, there is a need for caution in extrapolating the risk determinations based on a small number of individuals to whole communities and populations of native fauna.

There are a number of uncertainties associated with the algorithm (and assumptions) used to calculate the egg TEQs. The lipid content of birds can vary considerably between different species and among individual birds, depending on life-stage, habitat, feeding behaviour, dietary composition, and the health and condition of the bird. Reported fat levels in birds range from <1% to >30% for some migratory birds, before migration. The lipid content of bird's eggs (and fish eggs) also varies among individuals and between species. For example, Jarman et al., (1993) reported a lipid content in peregrine falcon eggs of between 1.9 and 6.2% (mean 4.32%). Kumar et al., (2002) reported a lipid content in Adelie penguin eggs of 10.5% (7.7-12.8%) and in south polar skua eggs of 9.1% (4.5-13.9%). For this ERA, a lipid content of 8% in eggs was assumed, however, this may be

too high for some birds, leading to an over estimation of egg concentrations, and too low for others, leading to an underestimation of exposure concentrations.

There is uncertainty associated with the EMR used to calculate egg concentrations in fish and birds. Limited data are available of the amount of maternal transfer of dioxin-like chemicals from adult female birds and fish to their eggs. These data are based on a limited number of species and congeners. Most of the data also predate recent developments and improvements in analytical methods used to measure dioxin-like chemicals. It is quite likely that the EMR will vary among bird and fish species, and even among individuals of the same species. Consequently, variability in the EMR could either under or over estimate egg TEQs.

There is significant uncertainity associated with the tissue concentrations predicted from soils and sediment data and the BSAFs. Much of the uncertainty is associated with the determining the actual range of exposures encountered by each animal. Within the areas where animals were found, only a few soil and sediment data points were available from which to determine average soil exposure concentrations. The NDP sampling survey strategy to avoid hotspots could have lead to an overestimation of the bioaccumulation factors (particularly in urban/industrial areas) if unusual local contamination existed in any of the animal's home ranges, but were not included in the calculation. Alternately, if unusually high levels were recorded in some area, which did not represent the average for that area, BSAFs could be underestimated, because the actual exposure would be exaggerated.

Extrapolation of BSAFs from one ecosystem to another to predict TEQs in organisms is associated with uncertainty. Adjustments for differing lipid and organic carbon content are built into the BSAF calculation and this reduces the uncertainty associated with site differences in organic carbon content, as well as variability in lipid levels in organisms.

The BSAF approach bypasses the intermediate, diet-mediated steps in bioaccumulation. Thus, there is considerable uncertainty associated with the relationship between soil concentrations and tissue level in prey items consumed by predators. Dioxin accumulation in individual raptors and between species of raptors, for example, will be influenced by differences in the temporal and spatial variability of levels in their prey. Sample et al., (1998) found accumulation based on the soil concentration greatest for insectivorous small mammals compared to herbivorous small mammals. This was attributed to food chain accumulation (consumption of herbivorous and predatory invertebrates). Thus, raptors consuming mainly insectivorous birds and mammals may be exposed to higher concentrations of dioxins than those consuming herbivorous prey.

A further complication in determining exposure is associated with the temporal variability in bioaccumulation. Theoretically, when organisms are continuously exposed to accumulating chemicals maintained at a fairly constant level in food, tissue concentrations will increase with time until, either a lethal concentration is reached and the organism dies, or a steady state is reached. The bioaccumulation factor at steady state (i.e. the rates of uptake and loss are equal) represents the highest value that can be reached, and therefore, indicates maximum risk for that organism (Walker, 2001). BSAFs measured before steady

state is reached are dependent on a range of factors including the intensity and frequency of exposure, and the age, gender, size and lipid levels. Because, the exposure history and individual details of each of the organisms are unknown, it is not clear whether the measured tissues concentrations represent steady state values for a given exposure regime or not. Individuals that move over a wide area or migrate seasonally, may encounter high exposure levels in pulses, interspersed by period of low exposure during which time some of the tissue residues may be metabolised. Such animals would require a longer time to reach a steady-state body burden.

Despite the uncertainties, BSAFs consistently give the most reliable estimates of fish tissue concentrations relative to other methods (e.g. BAF, BCF) (Pelka, 1998), with site specific and field derived BSAFs improving the accuracy of results considerably over data predicted from chemical properties and modeling. Thus, when tissue concentrations are not available, the BSAF approach is probably the best available method to predict exposure to dioxin-like chemicals on the basis of body burden. The BSAF is particularly useful for comparing relative differences in bioaccumulation between congeners, and for comparing relative bioaccumulation between different trophic levels.

## 4.8 Summary and conclusions

This chapter estimates the likelihood and severity of risk to Australian native aquatic and terrestrial organisms from exposure to dioxins at ambient levels. A tiered approach is adopted involving an initial screening level characterisation using the quotient method, followed by higher tiered characterisation using distribution curves of effects, and measured and predicted exposure concentrations.

A low risk to fish is indicated from exposure to ambient dioxin levels found in the Australian aquatic environment, when using the TRV for the most sensitive fish species tested.

A low risk to terrestrial mammals is also indicated from exposure to ambient levels of dioxins when assuming TRVs derived for placental mammals exposed during gestation. The absence of data on the toxicity of dioxins to native marsupials and monotremes add significant uncertainties to this risk determination, however, because the ramifications of the differing reproduction strategies between placental mammals and marsupials for dioxin exposure at sensitive life stages are not known.

Recognizing the inherent uncertainties in the models and assumptions, the data is sufficient to signal a potential risk to raptors exposed to ambient levels of dioxins, at least in the regions and subpopulations of birds from where the samples with the highest dioxins loads were collected. Approximately 30% of egg exposure concentrations were higher than the TRV for the most sensitive species - the domestic chicken, while approximately 20% of egg exposure concentrations were above the threshold for the bald eagle, and about 10% of eggs were above the threshold for embryo mortality in the American kestrel. Birds with the highest dioxin loads were collected in urban environments.

Despite these risk estimations, at the present time, there is no evidence for an unexplained decline in raptor populations in the wild in Australia, and no evidence that dioxin levels found in Australian wild raptors are causing reproductive and developmental impairment in the natural environment. The highest TEQ estimated in an Australian raptor egg from measured tissue concentrations was 245 pg/g egg, wet weight. Overseas field studies suggest that TEQs up to 126 pg/g do not impact hatching success, or cause edema, deformities, or changes in the renal and hepatic porphyrin levels of wild osprey, but may case increases in hepatic EROD activity, and affect the metabolism of vitamin A compounds. Levels up to 258 pg TEQ (based on the TEF system of Safe, 1990) in osprey eggs did not impact egg hatching and chick fledging rates, but resulted in reduced growth rates in chicks. It is not known, however, where osprey lie on the species sensitivity distribution curve, relative to other raptor species.

Based on a very small data set and limited toxicity information, a potential risk is indicated for the two dolphins living in the vicinity of urban/industrial estuaries, which had higher levels of dioxins in their bodies than aquatic mammals living in the open ocean. Tissue TEQ levels in these animals were within the threshold range TRVs found to cause toxic effects in laboratory and semi-field studies with mink, seal, and otters, which are the only aquatic mammalian wildlife for which TRVs are available. The risks to dolphins in other regions and dwelling in the open ocean are not known.

No risk is indicated for marine mammals living in the open ocean environments of Australia as these animals had body burdens well below the threshold TRV range.

The above risk estimations are based on a number of assumptions and are associated with many uncertainties and these should be taken into account when interpreting the risk. The confidence level that can be placed on risk estimations based on very small data sets and associated with large uncertainties and data gaps is somewhat low. Nevertheless, the body burden found in some individual raptors and dolphins are high enough to signal a cause for concern for sensitive early life stages potentially exposed to dioxins through maternal transfer.

A more reliable risk estimation of dioxins would require more information on the toxicity of dioxins to Australian wildlife species, and on the differences in sensitivity among species and classes of organisms. Australian ethical committees and current State legislation generally do not allow toxicity testing on native species. More targeted sampling of raptors and other high trophic level bird's eggs, performed in association with field population studies of potentially exposed birds, would help to clarify whether dioxins are having an impact on wild bird subpopulations.

The findings of this ERA also suggest that organisms at the top of the food chain, specifically raptors and dolphins, may accumulate relatively high loads of dioxins when exposed to ambient environmental levels. However, localised dioxin hotspots, not accounted for in this risk assessment, cannot be ruled out as a source for dioxins in some individual animals with high levels, particularly those collected in urban/industrial environments. This is because dioxins are highly persistent in soil and sediment, and therefore may still be present at high levels in some areas eventhough scheduled waste

initiatives are currently in place to limit releases of dioxins into the environment. Most existing contaminated sites are the result of historical rather than current emissions, arising from a time when there were few controls on dumping of hazardous wastes.

More information is required on the bioaccumulation and biomagnification potential of dioxins from ambient levels, particularly in terrestrial environments and high trophic level biota. Reliable bioaccumulation factors would enable risk assessors to predict the highest possible steady-state body burden that could be reached in vulnerable top predators under a given ambient exposure condition, and hence predict maximum possible risk. Reliable steady-state BSAFs would also enable better determination of safe threshold levels for setting soil and sediment quality guidelines.

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