


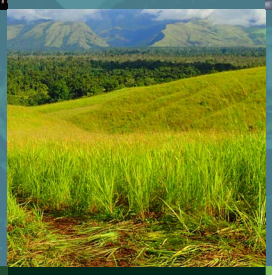


Appendix N

Assessment of Metal Bioaccumulation and Biomagnification from DSTP in the Huon Gulf

DISCLAIMER

This disclaimer applies to and governs the disclosure and use of this Environmental Impact Statement ("EIS"), and by reading, using or relying on any part(s) of the EIS you accept this disclaimer in full.

This Environmental Impact Statement, including the Executive Summary, and all chapters of and attachments and appendices to it and all drawings, plans, models, designs, specifications, reports, photographs, surveys, calculations and other data and information in any format contained and/or referenced in it, is together with this disclaimer referred to as the "**EIS**".

Purpose of EIS

The EIS has been prepared by, for and on behalf of Wafi Mining Limited and Newcrest PNG 2 Limited (together the "**WGJV Participants**"), being the participants in the Wafi-Golpu Joint Venture ("**WGJV**") and the registered holders of exploration licences EL 440 and EL1105, for the sole purpose of an application (the "**Permit Application**") by them for environmental approval under the Environment Act 2000 (the "**Act**") for the proposed construction, operation and (ultimately) closure of an underground copper-gold mine and associated ore processing, concentrate transport and handling, power generation, water and tailings management, and related support facilities and services (the "**Project**") in Morobe Province, Independent State of Papua New Guinea. The EIS was prepared with input from consultants engaged by the WGJV Participants and/or their related bodies corporate ("**Consultants**").

The Permit Application is to be lodged with the Conservation and Environment Protection Authority ("**CEPA**"), Independent State of Papua New Guinea.

Ownership and Copyright

The EIS is the sole property of the WGJV Participants, who reserve and assert all proprietary and copyright ©2018 interests.

Reliance and Use

The EIS is intended and will be made available to CEPA, for review by CEPA and other applicable agencies of the Government of the Independent State of Papua New Guinea ("**Authorised Agencies**"), for the purpose of considering and assessing the Permit Application in accordance with the Act ("**Authorised Purpose**"), and for no other purpose whatsoever.

The EIS shall not be used or relied upon for any purpose other than the Authorised Purpose, unless express written approval is given in advance by the WGJV Participants.

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This disclaimer must accompany every copy of the EIS.

The EIS is meant to be read as a whole, and any part of it should not be read or relied upon out of context.

Limits on investigation and information

The EIS is based in part on information not within the control of either the WGJV Participants or the Consultants. While the WGJV Participants and Consultants believe that the information contained in the EIS should be reliable under the conditions and subject to the limitations set forth in the EIS, they do not guarantee the accuracy of that information.

No Representations or Warranties

While the WGJV Participants, their Related Bodies Corporate and Consultants believe that the information (including any opinions, forecasts or projections) contained in the EIS should be reliable under the conditions and subject to the limitations set out therein, and provide such information in good faith, they make no warranty, guarantee or promise, express or implied, that any of the information will be correct, accurate, complete or up to date, nor that such information will remain unchanged after the date of issue of the EIS to CEPA, nor that any forecasts or projections will be realised. Actual outcomes may vary materially and adversely from projected outcomes.

The use of the EIS shall be at the user's sole risk absolutely and in all respects. Without limitation to the foregoing, and to the maximum extent permitted by applicable law, the WGJV Participants, their Related Bodies Corporate and Consultants:

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- expressly disclaim any liability for any consequential, special, contingent or penal damages whatsoever.

The basis of the Consultants' engagement is that the Consultants' liability, whether under the law of contract, tort, statute, equity or otherwise, is limited as set out in the terms of their engagement with the WGJV Participants and/or their related bodies corporate.

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The WGJV Participants acknowledge and agree that, for the Authorised Purpose, the EIS may be:

- copied, reproduced and reprinted;
- published or disclosed in whole or in part, including being made available to the general public in accordance with section 55 of the Act. All publications and disclosures are subject to this disclaimer.

Development of Project subject to Approvals, Further Studies and Market and Operating Conditions

Any future development of the Project is subject to further studies, completion of statutory processes, receipt of all necessary or desirable Papua New Guinea Government and WGJV Participant approvals, and market and operating conditions.

Engineering design and other studies are continuing and aspects of the proposed Project design and timetable may change.

NEWCREST MINING LIMITED DISCLAIMER

Newcrest Mining Limited ("**Newcrest**") is the ultimate holding company of Newcrest PNG 2 Limited and any reference below to "Newcrest" or the "Company" includes both Newcrest Mining Limited and Newcrest PNG 2 Limited.

Forward Looking Statements

The EIS includes forward looking statements. Forward looking statements can generally be identified by the use of words such as "may", "will", "expect", "intend", "plan", "estimate", "anticipate", "continue", "outlook" and "guidance", or other similar words and may include, without limitation, statements regarding plans, strategies and objectives of management, anticipated production or construction commencement dates and expected costs or production outputs. The Company continues to distinguish between outlook and guidance. Guidance statements relate to the current financial year. Outlook statements relate to years subsequent to the current financial year.

Forward looking statements inherently involve known and unknown risks, uncertainties and other factors that may cause the Company's actual results, performance and achievements to differ materially from statements in this EIS. Relevant factors may include, but are not limited to, changes in commodity prices, foreign exchange fluctuations and general economic conditions, increased costs and demand for production inputs, the speculative nature of exploration and project development, including the risks of obtaining necessary licences and permits and diminishing quantities or grades of reserves, political and social risks, changes to the regulatory framework within which the Company operates or may in the future operate, environmental conditions including extreme weather conditions, recruitment and retention of personnel, industrial relations issues and litigation.

Forward looking statements are based on the Company's good faith assumptions as to the financial, market, regulatory and other relevant environments that will exist and affect the Company's business and operations in the future.

The Company does not give any assurance that the assumptions will prove to be correct. There may be other factors that could cause actual results or events not to be as anticipated, and many events are beyond the reasonable control of the Company. Readers are cautioned not to place undue reliance on forward looking statements. Forward looking statements in the EIS speak only at the date of issue. Except as required by applicable laws or regulations, the Company does not undertake any obligation to publicly update or revise any of the forward looking statements or to advise of any change in assumptions on which any such statement is based.

Non-IFRS Financial Information

Newcrest results are reported under International Financial Reporting Standards (IFRS) including EBIT and EBITDA. The EIS also includes non-IFRS information including Underlying profit (profit after tax before significant items attributable to owners of the parent company), All-In Sustaining Cost (determined in accordance with the World Gold Council Guidance Note on Non-GAAP Metrics released June 2013), AISC Margin (realised gold price less AISC per ounce sold (where expressed as USD), or realised gold price less AISC per ounce sold divided by realised gold price (where expressed as a %), Interest Coverage Ratio (EBITDA/Interest payable for the relevant period), Free cash flow (cash flow from operating activities less cash flow related to investing activities), EBITDA margin (EBITDA expressed as a percentage of revenue) and EBIT margin (EBIT expressed as a percentage of revenue). These measures are used internally by Management to assess the performance of the business and make decisions on the allocation of resources and are included in the EIS to provide greater understanding of the underlying performance of Newcrest's operations. The non-IFRS information has not been subject to audit or review by Newcrest's external auditor and should be used in addition to IFRS information.

Ore Reserves and Mineral Resources Reporting Requirements

As an Australian Company with securities listed on the Australian Securities Exchange (ASX), Newcrest is subject to Australian disclosure requirements and standards, including the requirements of the Corporations Act 2001 and the ASX. Investors should note that it is a requirement of the ASX listing rules that the reporting of Ore Reserves and Mineral Resources in Australia comply with the 2012 Edition of the Australasian Code for Reporting of Exploration Results, Mineral Resources and Ore Reserves (the JORC Code) and that Newcrest's Ore Reserve and Mineral Resource estimates comply with the JORC Code.

Competent Person's Statement

The information in the EIS that relates to Golpu Ore Reserves is based on information compiled by the Competent Person, Mr Pasqualino Manca, who is a member of The Australasian Institute of Mining and Metallurgy. Mr Pasqualino Manca, is a full-time employee of Newcrest Mining Limited or its relevant subsidiaries, holds options and/or shares in Newcrest Mining Limited and is entitled to participate in Newcrest's executive equity long term incentive plan, details of which are included in Newcrest's 2017 Remuneration Report. Ore Reserve growth is one of the performance measures under recent long term incentive plans. Mr Pasqualino Manca has sufficient experience which is relevant to the styles of mineralisation and type of deposit under consideration and to the activity which he is undertaking to qualify as a Competent Person as defined in the JORC Code 2012. Mr Pasqualino Manca consents to the inclusion of material of the matters based on his information in the form and context in which it appears.

HARMONY GOLD MINING COMPANY LIMITED DISCLAIMER

Harmony Gold Mining Company Limited ("**Harmony**") is the ultimate holding company of Wafi Mining Limited and any reference below to "Harmony" or the "Company" includes both Harmony Gold Mining Company Limited and Wafi Mining Limited.

Forward Looking Statements

These materials contain forward-looking statements within the meaning of the safe harbor provided by Section 21E of the Securities Exchange Act of 1934, as amended, and Section 27A of the Securities Act of 1933, as amended, with respect to our financial condition, results of operations, business strategies, operating efficiencies, competitive positions, growth opportunities for existing services, plans and objectives of management, markets for stock and other matters. These include all statements other than statements of historical fact, including, without limitation, any statements preceded by, followed by, or that include the words "targets", "believes", "expects", "aims", "intends", "will", "may", "anticipates", "would", "should", "could", "estimates", "forecast", "predict", "continue" or similar expressions or the negative thereof.

These forward-looking statements, including, among others, those relating to our future business prospects, revenues and income, wherever they may occur in this EIS and the exhibits to this EIS, are essentially estimates reflecting the best judgment of our senior management and involve a number of risks and uncertainties that could cause actual results to differ materially from those suggested by the forward-looking statements. As a consequence, these forward-looking statements should be considered in light of various important factors, including those set forth in these materials. Important factors that could cause actual results to differ materially from estimates or projections contained in the forward-looking statements include, without limitation: overall economic and business conditions in South Africa, Papua New Guinea, Australia and elsewhere, estimates of future earnings, and the sensitivity of earnings to the gold and other metals prices, estimates of future gold and other metals production and sales, estimates of future cash costs, estimates of future cash flows, and the sensitivity of cash flows to the gold and other metals prices, statements regarding future debt repayments, estimates of future capital expenditures, the success of our business strategy, development activities and other initiatives, estimates of reserves statements regarding future exploration results and the replacement of reserves, the ability to achieve anticipated efficiencies and other cost savings in connection with past and future acquisitions, fluctuations in the market price of gold, the occurrence of hazards associated with underground and surface gold mining, the occurrence of labour disruptions, power cost increases as well as power stoppages, fluctuations and usage constraints, supply chain shortages and increases in the prices of production imports, availability, terms and deployment of capital, changes in government regulation, particularly mining rights and environmental regulation, fluctuations in exchange rates, the adequacy of the Group's insurance coverage and socio-economic or political instability in South Africa and Papua New Guinea and other countries in which we operate.

For a more detailed discussion of such risks and other factors (such as availability of credit or other sources of financing), see the Company's latest Integrated Annual Report and Form 20-F which is on file with the Securities and Exchange Commission, as well as the Company's other Securities and Exchange Commission filings. The Company undertakes no obligation to update publicly or release any revisions to these forward-looking statements to reflect events or circumstances after the date of this EIS or to reflect the occurrence of unanticipated events, except as required by law.

Competent Person's Statement

The Wafi-Golpu Joint Venture is an unincorporated joint venture between a wholly-owned subsidiary of Harmony Gold Mining Company Limited and a wholly-owned subsidiary of Newcrest Mining Limited.

The information in the EIS that relates to Golpu Ore Reserves is based on information compiled by the Competent Person, Mr Pasqualino Manca, who is a member of The Australasian Institute of Mining and Metallurgy. Mr Pasqualino Manca, is a full-time employee of Newcrest Mining Limited or its relevant subsidiaries, holds options and/ or shares in Newcrest Mining Limited and is entitled to participate in Newcrest's executive equity long term incentive plan, details of which are included in Newcrest's 2017 Remuneration Report. Ore Reserve growth is one of the performance measures under recent long term incentive plans. Mr Pasqualino Manca has sufficient experience which is relevant to the styles of mineralisation and type of deposit under consideration and to the activity which he is undertaking to qualify as a Competent Person as defined in the JORC Code 2012. Mr Pasqualino Manca consents to the inclusion of material of the matters based on his information in the form and context in which it appears.

Assessment of Metal Bioaccumulation and Biomagnification from DSTP in the Huon Gulf

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27 April, 2018

Revision History

Revision	Description	Date	Author	Reviewer	Signatory
Rev A	Draft	27/04/2018	Jerry Diamond (Tetra Tech)	Daniel Moriarty Ivan Steward Travis Wood (Coffey)	Daniel Moriarty (Coffey)
Rev B	Final	25/06/2018	Jerry Diamond (Tetra Tech)	Daniel Moriarty Ivan Steward Travis Wood (Coffey)	Daniel Moriarty (Coffey)

Client ref: 532-1208-EN_BioaccumulationStudy_RevD

Overall Conclusion

Using site-specific environmental data from the Huon Gulf (including biological and physical data collected for the Wafi-Golpu Environmental Impact Statement (EIS) study) and internationally accepted metal bioaccumulation modelling methods incorporating highly conservative assumptions, this study has found that the use of deep sea tailings placement (DSTP) for the Wafi-Golpu Project is predicted to not result in the elevation, above background levels, of metal burdens in fish consumed by people. The exception is manganese, which is predicted to increase in fish to a concentration double the background range. However, the predicted maximum manganese concentration of 0.241mg/kg in fish is an order of magnitude lower than daily concentrations required in the human diet and up to two orders of magnitude lower than concentrations where there are risks of adverse human health effects.

The study also found that DSTP is predicted to not cause any metals in edible fish to exceed food standards, although in the case of arsenic and mercury, some existing (i.e., prior to any DSTP) fish tissue concentrations in the Huon Gulf exceed food standards.

Executive Summary

Wafi Mining Limited and Newcrest PNG 2 Limited (WGJV Participants) are equal participants in the Wafi-Golpu Joint Venture (the WGJV). The WGJV is investigating the feasibility of constructing, operating and (ultimately) closing an underground copper-gold mine and associated ore processing, concentrate transport and handling, power generation, water and tailings management and related support facilities and services (hereafter the "Wafi-Golpu Project" or the "Project"), located beneath Mt Golpu, approximately 300 kilometres (km) north-northwest of Port Moresby and 65km south-west of Lae in the Morobe Province of the Independent State of Papua New Guinea (PNG). The Project includes ore processing, concentrate transport and handling, power generation, water management, a deep sea tailings placement (DSTP) system for tailings management, access roads to the mine and related support facilities.

The WGJV has commissioned a range of studies to inform the Project's Feasibility Study Update and to prepare an Environmental Impact Statement (EIS). This report describes the findings of the metal bioaccumulation and biomagnification study.

This study evaluates the potential for DSTP to lead to increased concentrations of metals of concern in fish that people may consume from the Huon Gulf. This report evaluates the bioavailability of metals in the tailings discharge and the biological pathways by which metals could accumulate in fish that people consume. The objectives of this evaluation are to:

1. Determine plausible pathways regarding the transfer of metals from the DSTP discharge to fish consumed by people given site-specific conditions.
2. Predict the likely range of metal concentrations in representative fish that people consume from the Huon Gulf as a result of DSTP discharge.
3. Determine whether metal concentrations in fish that people may consume would exceed Australian and New Zealand Food Safety standards (FSANZ).

The findings of this study informed a separate investigation of human health risks for the Project.

For this evaluation, the following metals were selected based on either their predicted concentrations in the Project mine tailings or because they are known potential threats to human health: arsenic, copper, nickel, mercury, manganese, and zinc.

There are three major pathways by which fish may theoretically accumulate metals from DSTP: (1) via metal accumulation in benthos that are in direct contact with the tailings deposited on the seafloor and trophic transfer up the food chain to fish consumed by people; (2) via metals accumulated in micronekton and zooplankton that are exposed to the dilute sub-surface tailings plumes, and then trophic transfer to fish consumed by people; and (3) via direct bioconcentration of bioavailable metals from the dilute sub-surface tailings plume into fish across their gills. This report evaluates each of these pathways using site-specific information collected from the Huon Gulf during EIS studies (including biological data such as metals concentrations in zooplankton, micronekton and fish) and metal bioaccumulation information from other DSTP and mine waste disposal sites in the Asia-Pacific region.

A range of Project-specific technical studies completed by Coffey and other sub-consultants and published sources from the Huon Gulf and PNG were used in this evaluation, as well as other published literature regarding metal bioavailability and bioaccumulation in marine food webs. Biological and geochemical data collected from the Huon Gulf were the primary source of information used to characterise the food web that could potentially be exposed to DSTP in the Huon Gulf. Project-specific DSTP modelling results and tailings testwork results were used to determine the bioaccumulation and biomagnification in the food web of the Huon Gulf. Information from DSTP sites in the Asia-Pacific region was used to support predictions regarding the fate of metals from DSTP discharge in the Huon Gulf and bioaccumulation estimates in fish consumed by people. Biological data on metals concentrations in fish tissues was also reviewed from EIS studies.

The Huon Gulf is characterised as having a steeply sloping canyon wall that reaches depths exceeding 2,000m within a fairly short distance from shore (0.2 to 5km offshore). It forms the westerly extremity of the New Britain Trench, with the broadening floor of the Markham Canyon feeding directly to the New Britain Trench. Much of the New Britain Trench is deeper than 4,000m and maximum depths exceed 9,000m. Monitoring at many locations in and around PNG suggests that the ocean water column is consistently stratified between the warm surface waters and the deep colder water. Following more than 12 months of upper ocean profiling (i.e., upper 500m of the water column), no evidence of strong stratification (i.e., sharp changes in temperature and density) has been observed in the Huon Gulf, and this is possibly related to surface wind-induced surface mixing and the frequent influx of river water and associated suspended sediments. However, the deep sea zone of the water column appears to be generally separate from upper surface water layers due to an apparent lack of upwelling. The absence of upwelling is supported by satellite imagery and from the results of upper ocean profiling of conductivity, temperature and density measured over an annual cycle in the Huon Gulf.

Examination of natural sediment originating from rivers near the proposed DSTP outfall demonstrates that the natural sediments descend rapidly down the slope of the canyon wall in the Huon Gulf, flowing as a density current and accumulating on the sea floor at depths beyond 2,000m.

Three-dimensional modeling of the predicted DSTP tailings discharge suggests that several subsurface plumes are predicted to shear off from the descending density current at depths between 300 and 500m, below which the majority of the tailings is predicted to descend as a bottom-attached density current along the seafloor. The modelling has shown that about 40% of the tailings is predicted to disperse in the water column as subsurface plumes, while the remainder would deposit on the canyon floor at depths in excess of 2,000m. The finer tailings are predicted by modelling to shear off laterally as dilute subsurface plumes between 300 to 500m depth reaching 1,800 dilutions within about 2.2km from the DSTP outfall. This number of dilutions is predicted to result in the tailings liquor complying with PNG ambient marine water quality criteria for all metal contaminants.

Benthic sampling (11 sites in February 2017) and analysis of video footage taken at the seafloor of the Huon Gulf (19 sites in December 2016) indicate that the benthos is generally very sparse and the benthic

habitat is covered by a thick layer of silts and clays originating from the nearby rivers. Riverine sediment is frequently transported through the Markham Canyon via episodic mass movements likely due to canyon wall slumping. Sampling of the floor of the canyon has shown a higher proportion of coarse material and thinner veneer of fine sediment, likely reflecting these episodic events. Chemical analysis of seafloor sediment indicated that metals would have low bioavailability to biota feeding on the natural sediments or fauna living in the sediments. Laboratory analysis using weak acid extraction techniques has shown metal bioavailability in Huon Gulf sediments to be low compared to sediment quality guidelines for protection of benthic biota. With the exception of copper, manganese and lead, bioavailable metals were at least an order of magnitude lower than the total metals concentrations. Bioavailable concentrations of copper, manganese and lead were between 5 to 30% of the total concentration. Low metal bioavailability is due to the naturally high sulphide concentrations present in the sediments, which transforms metals into insoluble metal compounds. In the tailings, much of the bioavailable metals concentrations are expected to be released in the dilute subsurface plumes. Laboratory testwork by the Commonwealth Scientific and Industrial Research Organisation (CSIRO) has shown that during the interaction of tailings/sediment mixtures with overlying seawater, the tailings continue to release concentrations of copper that exceed international ambient water quality guidelines into seawater for periods of up to 12 weeks. Despite the release of copper, the laboratory work found that the tailings/sediment mixture of 20/80 did not result in chronic or acute toxicity or bioaccumulation in test bivalve and amphipod species. An 80/20 tailings/sediment mixture was found to have no acute toxicity to amphipod survival but was found to result in chronic (reproductive) effects with amphipods. Testwork on the 80/20 tailings/sediment mixture using bivalve species was ongoing at the time of writing.

Results from upper ocean profiling conducted in the DSTP area between October 2016 and December 2017 indicates that upwelling does not occur. This indicates that deep waters in the Huon Gulf are disconnected from upper, warmer layers of seawater where fish are caught for consumption. Biological information collected from the Huon Gulf indicates that the fish consumed by people are part of trophic pathways that are largely disconnected from the food webs that occur near the seafloor. In the Huon Gulf, fish are caught to maximum depths of about 100m. This is because target species are mostly caught in these upper pelagic waters but also due to limitations of fishing equipment used. The disconnect between fish consumed by people and deep waters is also due in part to the lower depth limit (potentially as deep as about 500m) at which zooplankton and micronekton aggregations (the base of the food web for fish consumed by people) occur in the Huon Gulf. This is further supported by tissue metals data from the Huon Gulf and from other DSTP sites, which indicated little or no relationship between metals concentrations in benthic sediments and the food web, including fish which may be consumed by people. It is expected that there is limited uptake of metals by benthic biota from deep sea sediments because of the frequent mass movement events and highly dynamic nature of sediment movement in the Huon Gulf. Based on the evidence of the frequent mass movement events, it is therefore hypothesised that biota in the deep sea in the Huon Gulf would be continually adapting to and recolonising sediments, without long term exposure to any given sediment conditions.

Therefore, in the Huon Gulf there is unlikely to be a complete pathway by which metals from deposited tailings may bioaccumulate into fish consumed by people. The site conceptual model subsequently focuses on the DSTP subsurface plume pathways and considers both direct exposure of fish to metals from the DSTP subsurface plumes via bioconcentration across the fish gills, and indirect accumulation (bioaccumulation) of metals via ingestion of prey that have accumulated metals (Figure E-1).

Biological data collected from the Huon Gulf generally indicates a low diversity of fish near and below the DSTP outfall (from 200 to 1,000m). While over 30 species of fish were identified that are consumed by people, most of these species were caught by local fishermen from depths much shallower than the DSTP pipeline terminus, and mainly several nautical miles to the south of Lae. These species form the

pinnacle of a complex food web that comprises numerous zooplankton, micronekton (e.g., larval fish, and squid), and small (bait) fish species. Top trophic level fish that people consume (e.g., various tuna species including yellowfin, albacore, bigeye, and skipjack) have large home ranges and a varied diet and are therefore expected to have limited exposure to metals from subsurface DSTP plumes in the Huon Gulf. According to a fisheries and marine resource use study there are no known tuna spawning areas within the Huon Gulf, and no commercial tuna fisheries operate in the Huon Gulf. Based on the depth of the net drop needed to enclose fish (250m), it is also unlikely that the commercial purse-seine tuna fishery operating further offshore will target schooling tuna deeper than this 250m depth. Other fish species that are found near the seafloor, such as saddletail snapper, are not open ocean swimmers and, therefore, these species are not likely to be exposed to subsurface plumes in the water column. Deep-slope snapper species that would typically be expected to be present at depths where they would be exposed to tailings (i.e., on the deep-slope below about 200m) were not observed in the DSTP study area at depths greater than 200m during field studies and only shark species (which are not consumed by humans) were caught at such depths. The exception was one blackspotted croaker caught at 250m depth. Notwithstanding the lack of snapper species in the deep-slopes of the Markham Canyon, the snapper species caught at shallower depths and caught at least several kilometers from the DSTP study area are conservatively included in the food web model. Pelagic fish such as mahi-mahi, rainbow runner and narrow-barred Spanish mackerel are typically caught by trolling in surface waters, but their depth ranges are likely to extend into the water column where DSTP subsurface plumes are predicted to occur. Such species are included in the food web model. A simplified Huon Gulf food web diagram is shown in Figure E-2.

Baseline fish tissue metals data from a deep-slope and pelagic fish study in the Huon Gulf show that metal concentrations in fish currently consumed by people are below limits outlined in Australian and New Zealand food safety (FSANZ) standards except for arsenic and mercury. These two metals were found to exceed the FSANZ standards in many fish species consumed by people in the Huon Gulf, in the absence of DSTP. This assessment, which incorporated highly conservative assumptions in calculations, predicted that the concentrations of the metals of potential concern will generally not biomagnify to concentrations above background ranges in fish currently consumed by people in the Huon Gulf (Table E-1). The exception is that manganese is predicted to increase to twice the observed background range. This is due to the relatively high bioaccumulation factor for manganese at lower trophic levels. This maximum predicted concentration of 0.241mg/kg is low when compared to amount of manganese required in the human diet, which ranges from 1.2mg/day (in children) to 2.3mg/day (in adults). Furthermore, this maximum predicted manganese concentration is very low when compared to the literature reported daily dose above which health effects could occur (9.8mg per day). To exceed this value from fish consumption alone, one would need to eat in the order of 40kg of fish per day with a manganese concentration of 0.241mg/kg.

Figure E-1: Conceptual diagram depicting the major pathways by which metals from a DSTP discharge could potentially be accumulated in fish consumed by people in the Huon Gulf.

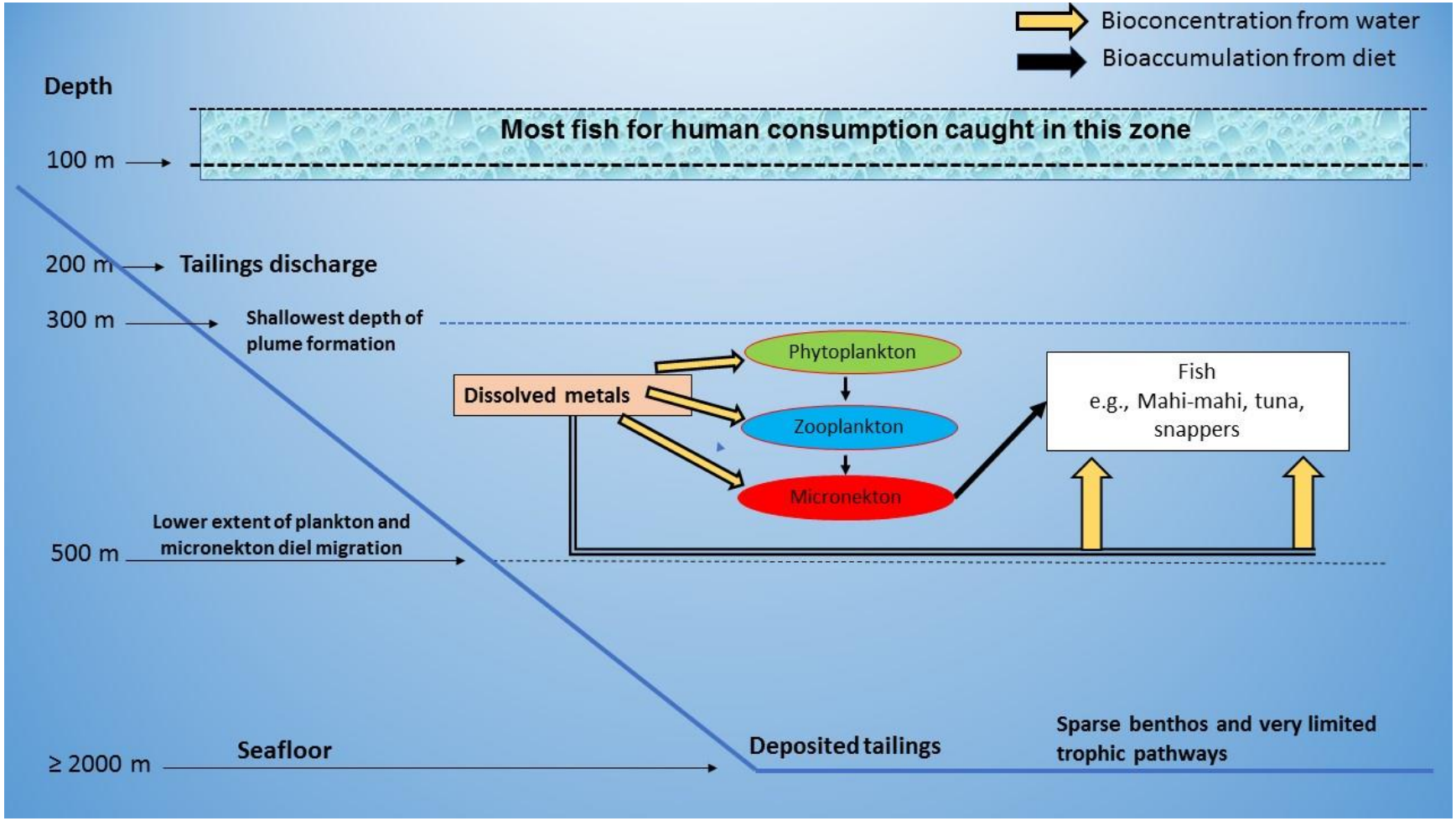


Figure E-2: Simplified food web diagram for some of the commonly consumed fish species in the Huon Gulf. Fish species noted in green are consumed by people in the Huon Gulf. Red arrows represent potential direct pathways between bottom-feeding fish and fish consumed by people.

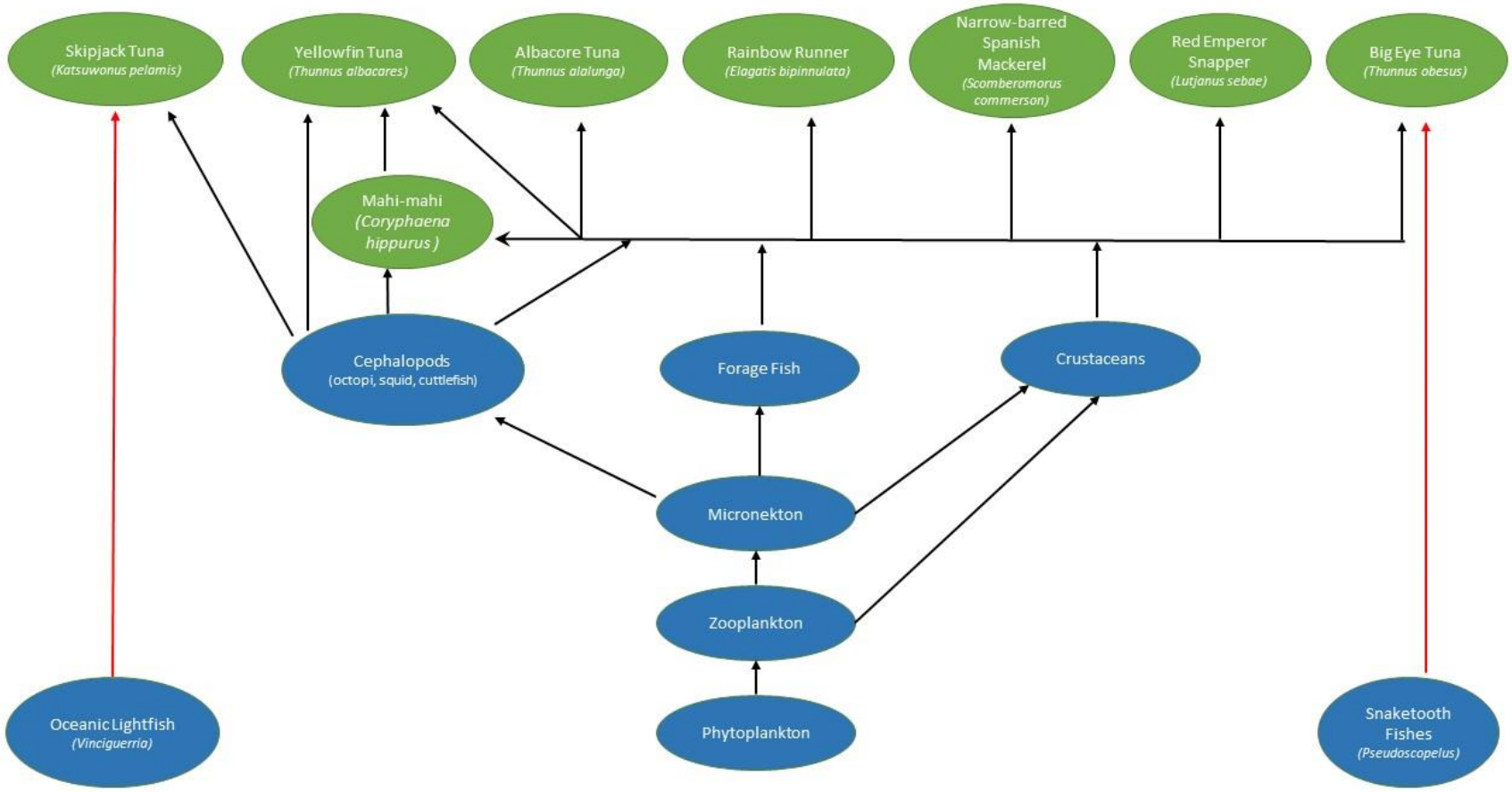
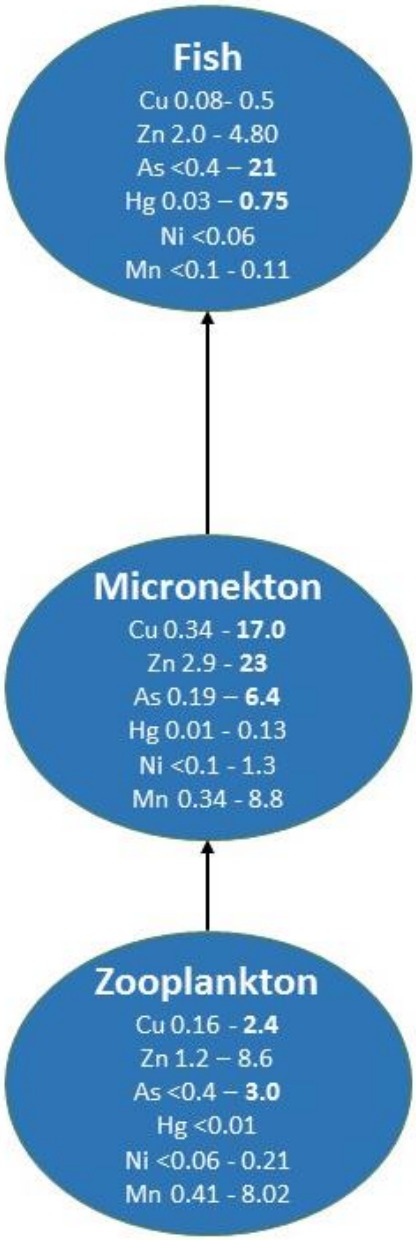


Figure E-3: Tissue metal concentrations (mg/kg wet weight) observed in three trophic levels (lowest trophic level at the bottom) of the Huon Gulf. Cu = copper, Zn = zinc, As = arsenic, Hg = mercury, Ni = nickel, Mn = manganese. Fish data are for muscle tissue and are based on species people consume, shark species are therefore not included. Bold values represent concentrations that exceed Australia and New Zealand food safety standards.



Predicted environmental metal concentrations were derived from DSTP modeling (Tetra Tech, 2018) and CSIRO elutriate testwork (CSIRO, 2018). The United States Environmental Protection Agency (USEPA) trophic pathway analysis methodology was used to incorporate both bioconcentration and bioaccumulation of metals in each trophic level (zooplankton, micronekton, and fish) based on species-specific ingestion rates and bioconcentration and bioaccumulation factors calculated using site-specific data in line with USEPA's methodology. The USEPA's food chain model used in this study is the standard procedure used by all federal and state regulatory agencies in the U.S., Canada, and other countries to predict chemical tissue concentrations in various aquatic species at potential contamination sites. For this screening analysis modelling, it was assumed that each trophic level and fish species was exposed to bioavailable metal continuously (i.e., there is no migration or movement away from the DSTP subsurface plume), which is a conservative assumption based on biological information collected from the Huon Gulf and other ocean sites in PNG.

Results of the screening analysis show that with the exception of arsenic, mercury, and copper, fish tissue concentrations of metals examined are unlikely to exceed FSANZ standards (Table E-1). The estimated fish tissue concentrations of these six metals (Table E-1), however, are similar to those measured at other DSTP sites in the region, indicating that the predictions are robust. Probable fish tissue metal concentrations due to DSTP are likely to be even lower than predicted in the screening analysis if observed movement patterns of biota in the Huon Gulf are considered (e.g., diel migrations of zooplankton and micronekton and habitat area use behaviours of upper trophic level fish).

Most of the fish species from the Huon Gulf that are consumed by people are unlikely to spend even 10% of their time in the DSTP area, let alone within a subsurface plume, because their preferred habitat and their prey live in other parts of the Huon Gulf. For this reason, it is also unlikely that more than 10% of the diet of fish would comprise zooplankton and micronekton that have been potentially exposed to the DSTP subsurface plumes. Therefore, the modelled concentrations in this study due to interaction with DSTP subsurface plumes are likely to be overestimated.

Using a conservative area use factor of 10% (0.1), the predicted fish tissue metals concentrations would be one tenth of those modelled. After applying an area use factor of 0.1, the results show that the presence of DSTP is predicted to result in no detectable change to metal concentration ranges already observed in fish from the Huon Gulf that are consumed by people (Table E-1).

Table E-1: Predicted DSTP subsurface plume dissolved metal concentrations and predicted metal tissue concentrations (mg/kg, wet weight) in representative fish consumed in the Huon Gulf, assuming a DSTP discharge. The table includes a comparison with observed fish tissue concentrations and food safety standards (FSANZ 2011). NGA = no guideline available. Bold values exceed food safety standards.

Metal	Predicted DSTP Subsurface Plume Concentration (mg/L)	Fish tissue metal Concentration from water ingestion only (mg/kg)	Fish tissue metal Concentration from prey ingestion only (mg/kg)	Maximum Predicted Concentration Considering Area Use Factor of 0.1 (mg/kg)	Existing Concentrations in Fish Consumed by People in the Huon Gulf (mg/kg)	FSANZ (mg/kg)
Arsenic	0.0016	**3.00**	**4.55**	0.455	<0.4 – **6.2**	2
Copper	0.011	**2.20**	**3.00**	0.3	0.11 – 0.50	2
Manganese	0.011	1.32	2.41	0.241	<0.1 – 0.12	NGA
Mercury	0.0000001	**0.53**	**0.53**	0.053	0.03 – **0.75**	0.5
Nickel	0.0014	0.07	0.087	0.0087	<0.06	NGA
Zinc	0.012	5.53	7.18	0.718	2.0 – 4.80	15

The predicted metals concentrations in fish do not include the very low background concentrations of metals in water. This omission does not appreciably affect the predictions for the six metals due to the very low background metals concentrations compared to the predicted metals concentrations in the DSTP subsurface plumes. Also, as the representative background tissue metals concentrations are calculated based on metals concentrations measured in the water (which were below detection limits), the maximum predicted concentrations in fish are due to DSTP only and not background concentrations in tissue.

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APPENDICES

Glossary

Artisanal fishery:	Small-scale fisheries for subsistence or local, small markets, generally using traditional fishing techniques and small boats.
Assimilation efficiency:	The efficiency by which animals convert the food they ingest into energy for growth and reproduction is called assimilation efficiency.
Bioavailable:	The extent to which a chemical can be absorbed by a living organism by active or passive processes and can react with cellular biochemical processes.
Bioaccumulation:	Increase in concentration of a pollutant from the environment to the first organism in a food chain.
Bioaccumulation factor (BAF):	BAF represents the ratio of the concentration of a chemical to its concentration in a medium. The factor must be measured at steady-state when the rate of uptake is balanced by the rate of excretion.
Bioconcentration:	A process by which there is net accumulation of a chemical directly from an exposure medium into an organism.
Bioconcentration factor (BCF):	BCF represents the ratio of the concentration of a chemical in an aquatic organism to the concentration of the chemical in surface water, sediment, or soil. The factor must be measured at steady-state when the rate of uptake is balanced by the rate of excretion.
Biomagnification:	Increase in concentration of a contaminant from one trophic level to another in a food chain.
Biomagnification factor (BMF):	The degree of trophic transfer.
Crustaceans:	A group of freshwater and saltwater invertebrates with jointed legs and a hard shell of chitin. Includes shrimps, crabs, lobsters, and crayfish.
Deep-slope:	Term used to describe animals associated with or living on the continental slope.
Density current:	A density current is the coherent movement of a mixture of seawater and suspended solids which, due to the high suspended solids concentration entrained in the mixture, is denser than the surrounding seawater. This density differential causes the density current to flow along the seafloor.
Depuration:	The loss of a compound from an ecological receptor as a result of any active or passive process.
DSTP:	Deep sea tailings placement.
DSTP Study Area:	The coastal and offshore benthic and pelagic environments of the upper Huon Gulf, broadly encompassing the lower reaches of the Markham and Busu Rivers, the Markham Canyon, and extending as far southeast as the upper reaches of the New Britain Trench.
EDTA:	Ethylenediaminetetraacetic acid.
Essential metals:	Metals that are required by organisms for normal metabolism.
Food web:	The interlocking patterns of food chains.

FSANZ:	Food Standards Australia New Zealand.
Micronekton:	Actively swimming organisms ranging in size between larger zooplankton (equal or more than 2cm) which drift with currents, and larger nekton (less than or equal to approximately 10cm), which have the ability to swim freely without being affected by prevailing currents.
Pelagic:	Term used to describe animals living throughout water column from surface to seafloor between the coastal zone and open ocean; used predominantly to describe fast swimming and/or schooling fishes found in the uppermost 200m, i.e., epipelagic zone.
Phytoplankton:	Algae that inhabit the water column.
Plume:	Refers to a concentration of suspended sediment as it spreads from its point of origin and moves: across the ocean surface (surface plume), as a submerged layer within the ocean water column (subsurface plume) or along the seafloor (bottom-attached plume).
PNG:	Independent State of Papua New Guinea.
Pycnocline:	Layer within the ocean in which water density increases rapidly with depth.
Species area use factor:	The spatial area typically inhabited by a species for foraging, reproduction, and normal behavior.
Trophic pathway:	Dietary linkages between organisms in different trophic levels (e.g., algae to zooplankton that eat algae).
Trophic level 1 (TL1):	Category of primary producer species such as algae and plants.
Trophic level 2 (TL2):	Category of primary consumer species (e.g., invertebrates) that directly forage on trophic level 1 species.
Trophic level 3 (TL3):	Category of secondary consumers (e.g., small fish) that directly forage on trophic level 2 species. These usually include fish that feed on invertebrates.
Trophic level 4 (TL4):	Category of tertiary consumers (e.g., larger fish) that directly forage on trophic level 3 species. These usually include fish that feed on small fish and large invertebrates.
Trophic transfer factor (TTF):	The ratio of metal concentration in predatory animals to metal concentration in prey organisms.
USEPA:	United States Environmental Protection Agency.
Zooplankton:	Animal component of plankton; mostly microscopic (equal or less than 2cm), free-swimming organisms found in the photic zone (layer of the ocean that receives sunlight; usually first 200m), and which drift with the prevailing currents.

1. Introduction

Wafi Mining Limited and Newcrest PNG 2 Limited (WGJV Participants) are equal participants in the Wafi-Golpu Joint Venture (the WGJV). The WGJV is investigating the feasibility of constructing, operating and (ultimately) closing an underground copper-gold mine and associated ore processing, concentrate transport and handling, power generation, water and tailings management and related support facilities and services (hereafter the "Wafi-Golpu Project" or the "Project"), located beneath Mt Golpu, approximately 300 kilometres (km) north-northwest of Port Moresby and 65km south-west of Lae in the Morobe Province of the Independent State of Papua New Guinea (PNG). The Project includes ore processing, concentrate transport and handling, power generation, water management, a deep sea tailings placement (DSTP) system for tailings management, access roads to the mine and related support facilities.

Geographically, the Project can be divided into three main areas, which together form the Project Area:

- Mine Area. The area encompassing the proposed block cave mine and nearby infrastructure, including the portal terrace, Watut and Nambonga declines, waste rock dump, Watut Process Plant, any power generation facilities, laydown areas, water treatment facilities, , wastewater discharge and raw water make-up pipelines, raw water dam, sediment control structures, roads and accommodation facilities for the construction and operations workforces.

- Infrastructure Corridor. The area encompassing the Project infrastructure linking the Mine Area and the proposed Coastal Area, being corridors for pipelines, roads and laydown areas. The proposed concentrate pipeline, terrestrial tailings pipeline and fuel pipeline will connect the Mine Area to the Coastal Area. A proposed Mine Access Road and Northern Access Road will connect the Mine Area to the Highlands Highway. New single-lane bridges are proposed over the Markham, Watut and Bavaga rivers. Laydown areas will be located at key staging areas.

- Coastal Area. The Coastal Area includes the proposed Port Facilities Area and the proposed Outfall Area:

    - Port Facilities Area. Located at, or in proximity to, the Port of Lae, with a site adjacent to Berth 6 (also known as Tanker Berth) nominated as the preferred option. The proposed facilities will include the concentrate filtration plant and materials handling, storage, ship loading facilities and filtrate discharge pipeline.

    - Outfall Area. Located approximately six kilometres east of the port. The proposed facilities will include the Outfall System comprising the mix/de-aeration tank and associated facilities, seawater intake pipelines and DSTP outfall pipelines, pipeline laydown area, choke station, access track and parking turnaround area..

The WGJV has commissioned a range of studies to inform the Project's Feasibility Study Update and to prepare an Environmental Impact Statement (EIS).

This report describes the findings of the metal bioaccumulation and biomagnification study. Analysis of biomagnification factors by Brewer et al. (2012) in different trophic levels at the Lihir gold mine found little evidence of widespread biomagnification from lower (zooplankton) to higher trophic levels (large pelagic fish). For most trace metals analysed, there was either decreasing concentrations of trace metals with increasing position in the food web, or some trophic transfer in the lower to mid-levels only (zooplankton

to micronekton and baitfish). Overall, very few differences were observed in the metal concentrations of micronekton and pelagic fish between mine and reference regions.

An assessment of metal bioaccumulation in fish for a jarosite dumping operation found that although there was the possibility of food web contamination in lower level predators (represented by squid), the increases in heavy metals concentrations in top predators such as southern bluefin tuna from the jarosite disposal were all relatively low, even under the worst-case assumptions (CSIRO, 1994).

Due to the importance of such potential impacts and the level of stakeholder interest, investigating potential linkages between DSTP and metal concentrations throughout the food web (including fish consumed by people) will inform the DSTP impact assessment as part of the Project EIS.

There are three major pathways by which fish may theoretically accumulate metals from DSTP: (1) via metal accumulation in benthos that are in direct contact with the tailings and trophic transfer up the food chain to fish consumed by people; (2) via metals accumulated in micronekton and plankton that are exposed to the tailings plume, and then trophic transfer to fish consumed by people; and (3) via direct bioconcentration of metals from the DSTP plume into fish across their gills. This report evaluates each of these pathways using site-specific information collected from the Huon Gulf, metal bioaccumulation information from other DSTP sites, and published literature regarding bioaccumulation and trophic transfer of select metals of concern.

A considerable amount of relevant data has already been collected from the Huon Gulf, including that regarding resident biota (Coffey, 2018a; 2018b; 2018c; 2018d; 2018e); fish consumed by people (Coffey, 2018c; 2018d; 2018e); metal concentrations in fish and potential prey items (Coffey, 2018b; 2018d), metal concentrations and physicochemical characteristics of simulated tailings and tailing liquor (CSIRO, 2018); and predicted hydrological characteristics of tailings discharged via DSTP into the Huon Gulf (Tetra Tech, 2018). These data and other information collected from the site are instrumental in predicting potential metal bioavailability and bioaccumulation given proposed DSTP, and ultimately predicting metal concentrations in fish from the Huon Gulf consumed by people.

This report addresses the following objectives: (1) identify plausible pathways given site-specific conditions of the proposed DSTP; (2) determine the degree of metal bioaccumulation that is probable in fish consumed by people given the likely pathway(s) and site-specific conditions; and (3) determine metal concentrations expected in fish and how they compare to existing tissue metals concentrations in fish and Australian and New Zealand food safety standards.

2. Site Conceptual Model

2.1. General Considerations

A site conceptual model is useful for communicating pathways by which particular pollutants may be taken up and transferred through the food chain given a particular source of the pollutants and the ecological setting. For this report, focus was placed on identifying viable pathways by which metals may bioaccumulate in fish consumed by people due to potential DSTP in the Huon Gulf. Viability of a given pathway (i.e., whether the pathway can be considered "complete") is determined by evaluating several site-specific factors including: potential transport and fate of metals from the tailings discharge; habitats of biota and trophic relationships between species in the Huon Gulf; and diet and habitat of fish species consumed by people and the degree to which their prey could be exposed to bioavailable metals originating from DSTP.

The following evaluates these three factors for building a site conceptual model and identifying the major pathway(s) for potential DSTP contaminants to pass through the food web in the Huon Gulf.

2.2. Fate and Transport of Metals from Proposed DSTP Project

Along with terrestrial options the Project is considering a deep sea tailings discharge facility to the Huon Gulf near Lae, PNG (Figure 2-1). The bathymetry in the northwestern region of the Huon Gulf comprises steep nearshore seabed slopes along with the occurrence of submarine canyons (in excess of 3,000m) in relatively close proximity to the shoreline. The Huon Gulf forms the westerly extremity of the New Britain Trench, with the broadening floor of the Markham Canyon feeding directly to the New Britain Trench. Much of the New Britain Trench is deeper than 4,000m and maximum depths exceed 9,000m (Coffey, 2018a; IHAconsult, 2018a).

Tailings are proposed to be discharged below the surface at 200m water depth. Upon entering the marine environment, the tailings are predicted to mix with and entrain seawater such that by the time the solids are deposited on the ocean floor, the tailings would be greatly diluted by seawater (CSIRO, 2018).

The deep sea bed has very weak interactions with the surface and coastal zones in PNG, based on extensive monitoring information and oceanographic information (SAMS, 2010). The ocean water column in the Huon Gulf is usually stratified between the warm surface waters and the deep colder waters (IHAconsult, 2018a). However, in oceanographic measurements collected further offshore, there has been no indication to date of strong water column temperature stratification to a depth of 500m; i.e., no persistent layers of water with strongly different density, salinity and/or temperature profiles. (Coffey, 2018b; IHAconsult, 2018b). IHAconsult (2018b) identified that a variable surface mixed layer is present with a maximum recorded depth of 96m and that this is overlying a strong and variable thermocline zone to a depth of 600m. This surface mixed layer depth was defined as the depth at which the temperature was 1°C less than the temperature at 10m depth and this definition has been employed for other DSTP projects in PNG (IHAconsult, 2018b).

DSTP modelling shows that the majority of the tailings (60%) is predicted to descend down the steep canyon slope as a bottom-attached density current and deposit on the seafloor. Examination of natural sediments originating from the rivers discharging to the upper western Huon Gulf shows that they also descend rapidly down the slope of the canyon and accumulate on the deep seafloor (IHAconsult, 2018a; Coffey, 2018a). Information collected at other DSTP sites in the region, such as Lihir (Brewer et al., 2007; 2012) and Batu Hijau (GESAMP, 2016), supports this observation, indicating that the tailings is predicted to flow as a density current to the seafloor.

Three-dimensional dispersion modeling of the DSTP discharge predicts that subsurface tailings plumes are predicted to shear off from the density current at depths between 300 and 500m (Figure 2-3), which is well below the maximum recorded surface mixed layer depth of 96m. The modelling shows that for 95% of the time, 1,800 dilutions – the dilutions required for the tailings liquor metals concentrations to comply with PNG ambient marine water quality criteria – is predicted to be achieved within about 2.2km of the DSTP outfall.

Figure 2-1: Physiographic setting of the Huon Gulf (IHAconsult, 2018a)

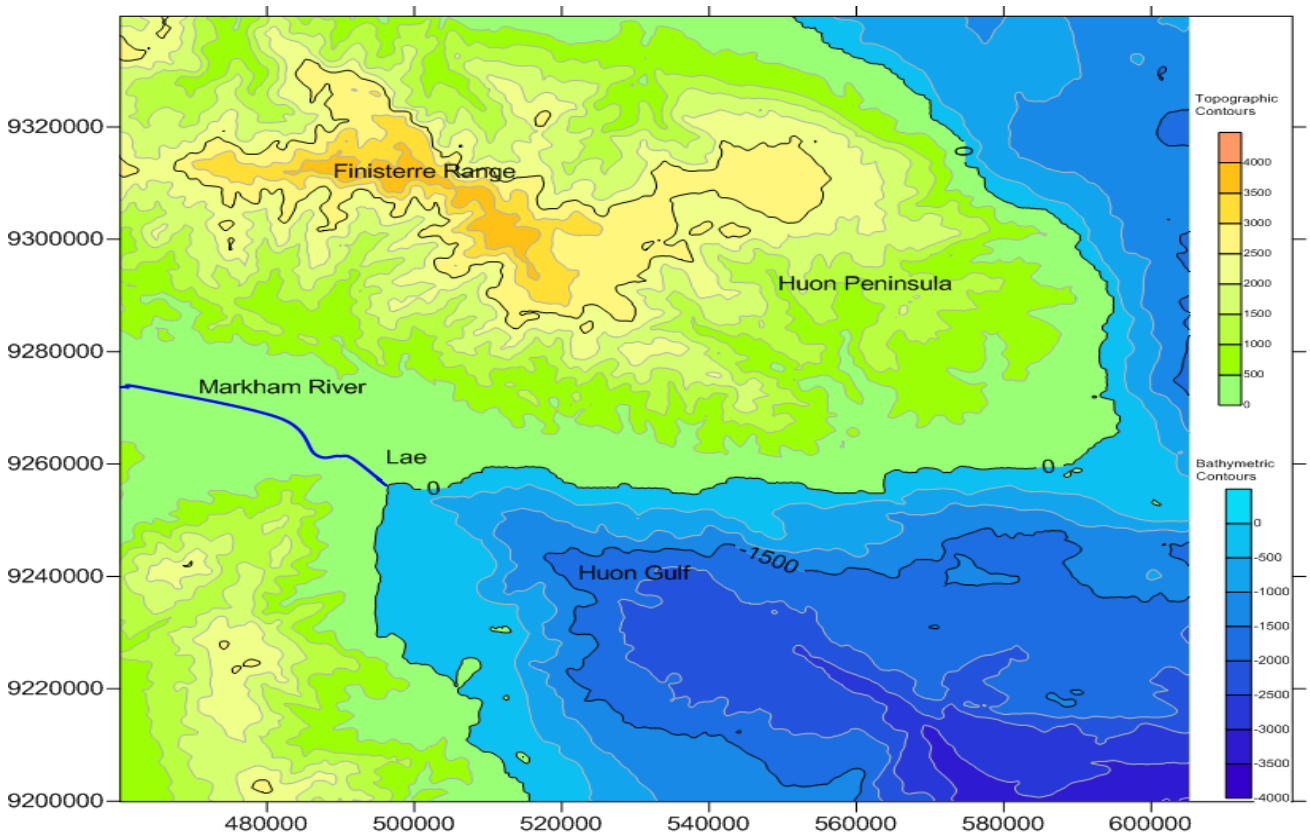
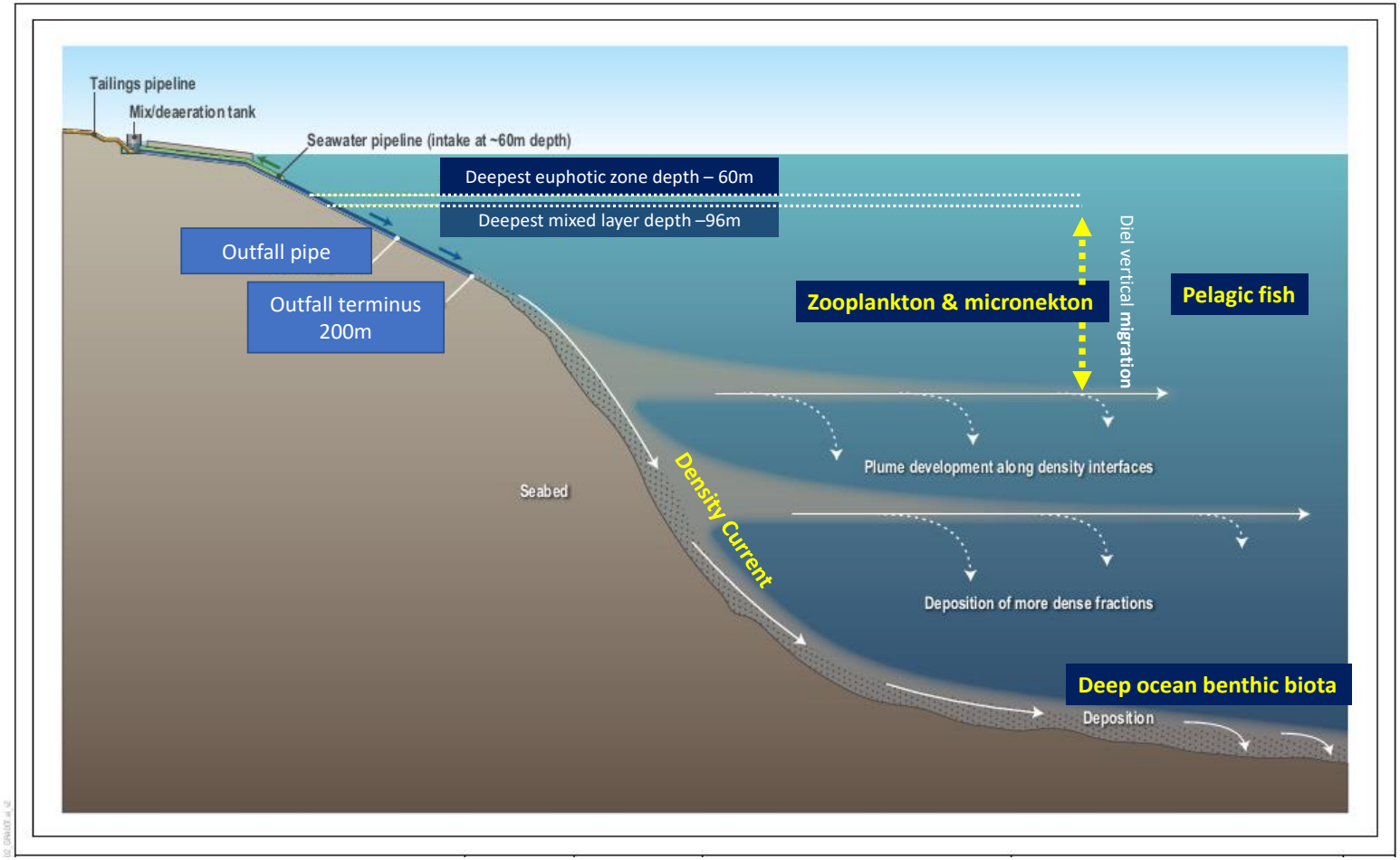


Figure 2-2: Conceptual depiction of the key interactions of DSTP with the food web at the proposed Project site in the Huon Gulf.



The subsurface plumes are not expected to be transported to shallower depths or mix with water closer to the surface (Figure 2-3) because of density differences between the depths at which they form and the upper water column, and the absence of upwelling or currents that would otherwise tend to push plumes upward in the water column (IHAconsult, 2018a;).

Studies of DSTP plumes at active sites such as Batu Hijau support these results, indicating that subsurface tailings plumes do not mix with seawater at depths shallower than the outfall (GESAMP, 2016). Acoustic sensing supported by direct measurements and water sampling of DSTP discharges at other sites in the Asia-Pacific region have shown that some of the tailings material separates from the descending density current and spreads out horizontally at density discontinuities in the water column. These subsurface plumes are dispersed laterally by currents, but remain trapped at depth by the ocean's natural density stratification. For the Project, these plumes are predicted to extend up to a horizontal distance of approximately 2.7km (where 5,000 dilutions is predicted to be reached) and become progressively more dilute with increasing distance from the DSTP outfall as demonstrated in modeling shown in Figure 2-3.

For the bioaccumulation modeling in the present study, the metal concentrations reported by CSIRO (2018) in elutriates of two tailings samples at 1:1,000 dilution were used. The elutriates were prepared by vigorously mixing each tailings sample with clean seawater for 12 hours at 30˚C in the laboratory (to replicate the mixing of the DSTP discharge in the receiving marine waters). After 12 hours, the elutriate samples were filtered (0.45µm) and dissolved metal concentrations were measured in the water fraction. As a conservative approach, for each metal the highest concentration for two tailings samples, Tailings 1 and Tailings 2, measured at 1:1,000 dilution was adopted as the metal concentration in the subsurface tailings plume. Mercury was not analysed in the CSIRO elutriate tests, but this metal is expected to be below detection limits in the undiluted tailings liquor (i.e., below 0.1µg/L) (Watt, pers comm. 2018) and this concentration was assumed in the assessment.

The highest modelled concentration contour of the tailings subsurface plumes, to which biota could be exposed, is 1:1,000 to 1:1,800 dilutions (Figure 2-3). Dilutions less than 1,800 are predicted to result in cobalt from the tailings liquor exceeding PNG ambient marine water quality criteria. Other metals meet water quality criteria at lower dilutions. Dispersion modelling has shown that 1,800 dilutions of the tailings subsurface plumes (liquid fraction) will be achieved 2,174m from the DSTP outfall 95% of the time.

While the CSIRO (2018) elutriate study conducted analyses up to a 1:10,000 dilution, it did not specifically include analysis of a 1:1,800 dilution. For this assessment, the metal concentrations at a 1:1,000 dilution (which was examined in the elutriate tests by CSIRO, [2018]) were assumed as the metal concentrations to which biota could be exposed. This 1:1,000 dilution is a conservative concentration given that it is at the most concentrated limit of the dilution contour between 1,000 to 1,800 dilutions.

2.3. Predicted Bioavailability of Metals in the Huon Gulf

Preliminary laboratory testing by CSIRO suggested that certain metals (e.g., copper, zinc, nickel) in tailings from the Wafi-Golpu Project may be released as demonstrated through repeated washings with seawater and in elutriates (CSIRO, 2018). Metal release was observed within a 24-hour period and additional testing indicated an increase in metal concentration in washings over a 72-hour period using laboratory procedures. Furthermore, some of the metals were bioavailable as evidenced by toxicity of elutriates and tailing liquor (tailings diluted by a factor of 4 with clean seawater; i.e., one part tailings to three parts seawater) to biota of different trophic levels including zooplankton, other crustacea, bivalves, echinoderms, and one fish species Yellow-tail Kingfish (*Seriola lalandi*) using standard laboratory chronic tests (CSIRO, 2018). Ecotoxicity testing, by exposing the tailings to eight bioassays from these different trophic levels, found that for two tailings samples (each with a different porphyry to metasediment ratio),

430 dilutions and 1,053 dilutions were required to result in protection of 95% of marine species, with 50% confidence.

Field information collected from the Huon Gulf, as well as from current DSTP sites in PNG and the Asia-Pacific region is useful for placing the laboratory studies into context. The Huon Gulf receives surface water from several major rivers in the vicinity of the proposed Wafi-Golpu Project (Figure 2-4). These rivers are high in suspended sediments and turbidity (IHAconsult, 2018a). High turbidity and suspended solids were recorded at many locations in the Huon Gulf benthic video study at and below the proposed tailings discharge point (Coffey, 2018a). The sedimentology study (IHAconsult, 2018a) indicated that much of the suspended sediment that is discharged to the Huon Gulf from the rivers is made up of fine silt and clay particles. These particles tightly adsorb cationic metals such as copper, nickel, and zinc (USEPA, 2007). Thus, metals such as copper, nickel, and zinc may not be as bioavailable in a DSTP plume at this site as predicted based on laboratory testing.

Metals data have been collected from sediments in the Huon Gulf that have primarily originated from riverine discharges. These data support the relatively low bioavailability of metals in the Huon Gulf, as indicated by the low EDTA-extractable metal concentrations as compared to Australia and New Zealand sediment quality guidelines, and also lower than the total metal concentrations (often by an order of magnitude or more) (IHAconsult, 2018a). Of the metals of interest in this study, only copper and manganese had measurable concentrations of potentially bioavailable metal in natural sediment (IHAconsult, 2018a). All EDTA extractable concentrations were below the Sediment Quality Guideline Value (SQGV) trigger levels, were similar to literature-reported mean crustal abundance values for these metals, and were generally less than metal values in deep sea clays (IHAconsult, 2018a).

The concentration of certain metals (e.g., copper) could be relatively high in the proposed Project tailings, including both reactive and inert mineralised forms (CSIRO, 2018). High sulphur and iron concentrations in the tailings (average of 20g/kg and 50g/kg dry weight, respectively) (CSIRO 2018), for example, can form sulphide and iron sulphide complexes with metals which will alter metal speciation and limit transport and bioavailability of metals. Most transition metals (e.g., copper, cadmium, nickel, and mercury) react readily with dissolved sulphide or particulate amorphous iron sulphides (both of which are abundant in the geological minerology of the mine site) to form highly insoluble (and low toxicity) metal sulphides (Chapman et al., 1998).

Figure 2-3: Results of 3-dimensional modeling of predicted subsurface tailings plumes given a proposed discharge depth of 200m. The figure shows total dilutions, which include any pre-discharge dilution with seawater.

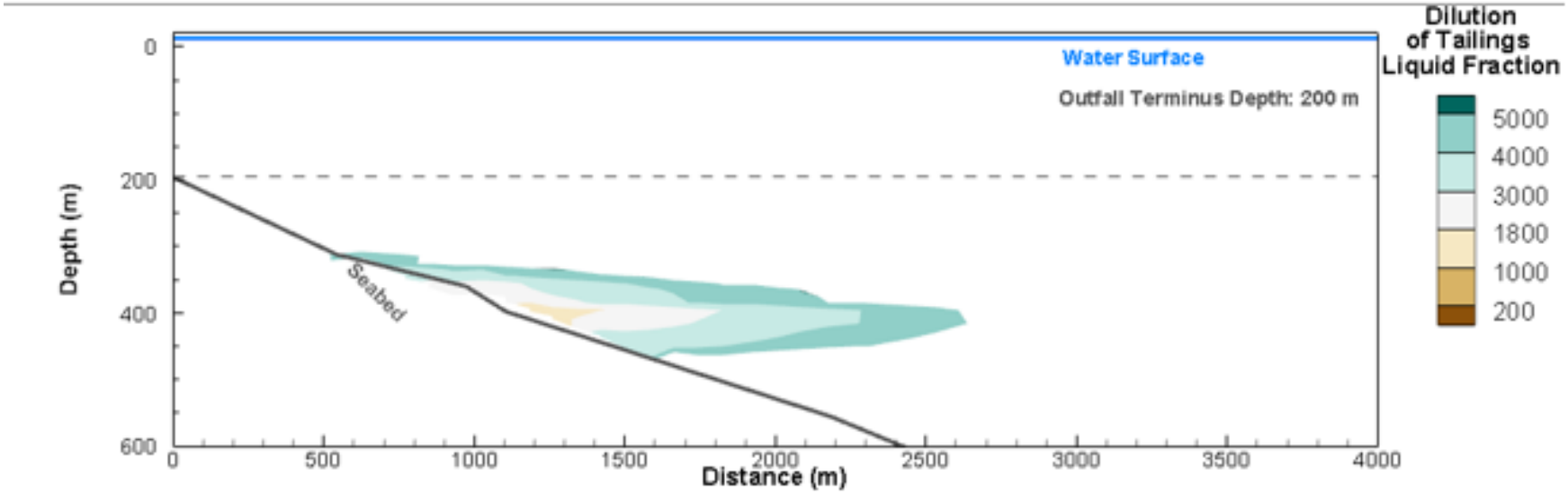


Figure 2-4 Rivers draining to the northern shoreline of the Huon Gulf (source: IHAconsult, 2018a).

2.4. Biota in Huon Gulf and Trophic Relationships

Zooplankton and micronekton communities recorded in the DSTP area were found to be of typical healthy marine systems (see Appendix A). However, the diversity and abundance of pelagic and deep-slope fish in the DSTP area were found to be very low (Coffey, 2018d). The following is a brief summary of the available information as it relates to the evaluation of biological pathways and potential bioaccumulation of metals in fish consumed by people.

Thirty-eight zooplankton taxa groups have been identified during the March 2017 survey (Figure 2-5; Coffey, 2018b). Of these, 32 taxa groups (84%) were common to all sites, depths, and sampling times. Taxa composition identified in zooplankton assemblages from the DSTP and reference study areas are indicative of a healthy community typical of tropical marine waters (i.e., numerous, diverse taxa with no one dominant taxon). The zooplankton community of the Huon Gulf was dominated by crustaceans, including copepods, ostracods and the, ghost shrimp Lucifer a decapod), which cumulatively accounted for 57% to 90% of the total number of taxa collected across all sampled sites.

Recent observations suggest that there is a diel migration pattern of zooplankton and micronekton aggregations in the Huon Gulf (Coffey, 2018b). This study indicated that there was vertical migration of zooplankton from deeper to shallower waters at night as evident from the higher abundances of zooplankton in night time samples taken from shallower depths. Factors which may play a role in driving the extent of vertical and horizontal distribution of zooplankton in the DSTP study area include daily tides, local and wind-driven ocean currents, turbidity of waters, and variable riverine discharge (and associated nutrient inputs) in the upper Huon Gulf. The lowest vertical limit of zooplankton distribution in the upper Huon Gulf is likely to be closely driven by maximum euphotic zone depth (i.e., which receives enough sunlight to allow photosynthesis to support phytoplankton communities). The maximum euphotic zone depth has been determined during a separate oceanographic study to be 60m (IHA consult, 2018b). Micronekton aggregations, however may extend deeper into the water column at depths of up to 500m (Coffey, 2018b).

Zooplankton data collected at other sites in PNG (e.g., Brewer et al., 2012) as well as in Indonesia (Morello et al, 2016) indicate that zooplankton and micronekton migrate vertically in the water column daily, with much of the zooplankton species concentrated between 100 and 600m deep during the daytime and in the uppermost 100m during the nighttime. Vertical movements of organisms near the base of the food web may be a key structuring component of the trophic dynamics in areas such as the Huon Gulf. Micronekton and small fish distribution patterns have been reported to follow the diel migration patterns of plankton, which in turn influences the vertical movement patterns of top predator fish such as tuna (Morello et al., 2016). The resulting patterns of diel movement have been viewed as a “conveyor-belt effect” (Morello et al., 2016), by which the upper layers of the water column may be linked by means of daily vertical movements of different species.

Micronekton is a group of various species that feed on zooplankton and includes decapods (e.g., shrimp), jellyfish, other small invertebrates, and larval fish. A diverse community of micronekton have been sampled from Huon Gulf (Figure 2-6; Coffey, 2018b). Micronekton, like zooplankton, have been shown to demonstrate diel vertical migration in the water column. In the Huon Gulf surveys, micronekton occurred at the offshore site from depths between the maximum sampling depth of 500m and the sea surface, and at the inshore site between 250m and the surface (Coffey, 2018b). The greatest micronekton abundance was recorded inshore between 0 and 250m depth at night. The micronekton assemblages comprised mostly chaetognaths, copepods, siphonophores, decapods and fishes.

Figure 2-5: Summary of major types of zooplankton recorded in the Huon Gulf (Coffey, 2018b).

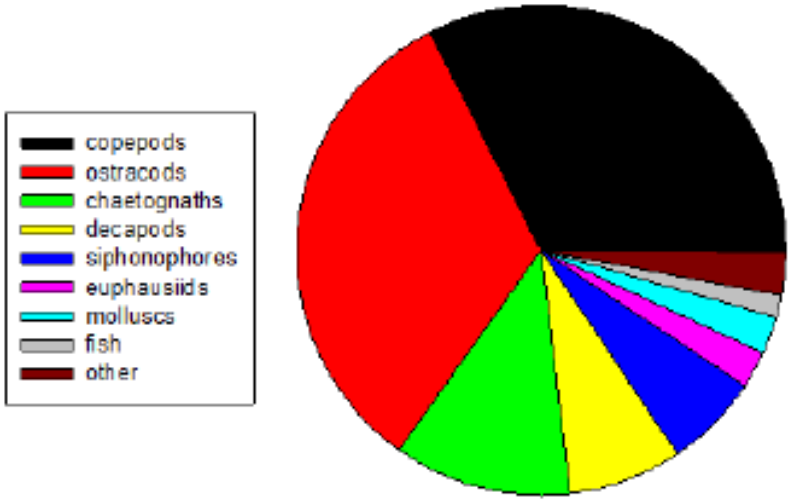
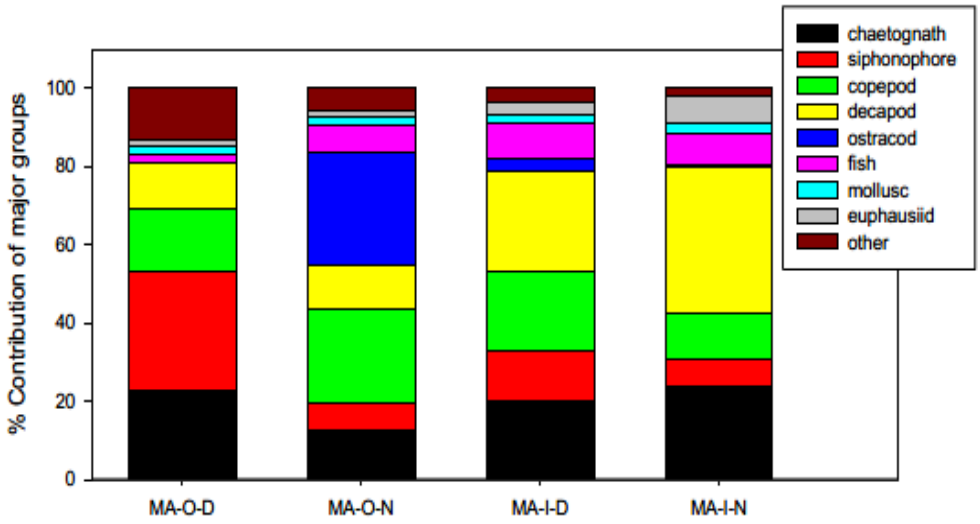


Figure 2-6: Percentage contribution of major micronekton groups identified from samples collected in the DSTP study area in the Huon Gulf in May 2017 (Coffey, 2018b).



'Other' category represents thaliaceans, appendicularians, amphipods, cnidarians and polychaetes

Figure notes: MA-O-D denotes micronekton sampled from an offshore site at depths between 500m and the surface during the day. MA-O-N denotes micronekton sampled from an offshore site at depths between 500m and the surface during the night. MA-I-D denotes micronekton sampled from an inshore site at depths between 250m and the surface during the day. MA-I-N denotes micronekton sampled from an inshore site at depths between 250m and the surface during the night.

Zooplankton and micronekton form the main diet of over 36 species of finfish that are harvested from the Huon Gulf coastal waters and used as the primary source of protein for local residents (Coffey; 2018c). Figure 2-7 illustrates some of the food web pathways that support a few of the widely consumed fish species in the Huon Gulf.

The diversity and abundance of pelagic and deep-slope fish in the DSTP area was found to be very low during field investigations, primarily comprising dwarf gulper sharks (*Centrophorus atromarginatus*) (Coffey, 2018c). Except for a single saddletail snapper (*Lutjanus malabaricus*; family Lutjanidae), no other deep-slope snappers or individuals from the other two most prevalent fish families recorded in comparable baseline studies elsewhere in PNG, namely Serranidae (sea basses and groupers) and Lethrinidae (emperors), were captured during the surveys in the DSTP area in November 2016 or May 2017. However, due to observations of these species being sold at local fish markets in Lae, species that may occur in the Huon Gulf include mangrove Jack (*Lutjanus argentimaculatus*), blackspotted croaker (*Protonibea diacanthus*), mackerel tuna (*Euthynnus affinis*), sharptooth jobfish (*Pristipomoides typus*), bigeye trevally (*Caranx sexfasciatus*) and the common pike eel (*Muraenesox bagio*) (Coffey, 2018d)

Four species of tuna (yellowfin, albacore, bigeye, and skipjack) are routinely fished by commercial fishers in offshore pelagic waters and are important economically. This fishing occurs more than 100 nautical miles outside the Huon Gulf, and there are no known tuna spawning areas within the Huon Gulf (EnviroGulf, 2017). The Food and Agriculture Organization (FAO) (2003) also notes that a maximum target depth of 250m is given for the commercial purse-seine tuna fishery based on the depth of the net drop needed to enclose the fish. In this way, the fishery typically targets the schooling tuna within the surface mixed layer rather than this 250m depth. There are several areas where tuna and tuna-like fish including wahoo (*Acanthocybium solandri*), blue marlin (*Makaira nigricans*), Spanish mackerel (*Scomberomorus commerson*) and yellowfin tuna are caught via surface trolling by local fishers, however the closest of these areas to the DSTP outfall is the Benalla Banks, which is a shallow area located 6.5km northwest of the Salamaua Peninsula and 25km south of the DSTP outfall (EnviroGulf, 2017). Tuna species are known to dive into deeper waters to search for prey; and while consequent exposure to tailings plumes is likely to be minimal, this does potentially serve as a means to transmit material from lower pelagic depths upward into the upper pelagic zone. One such potential vector (noted with red arrows in Figure 2-7) is that from deep sea finfish (e.g., *Pseudosopelus* spp. and *Vinciguerria* spp.) that have been documented to be significant components of big eye tuna and skipjack tuna diet, respectively (Kornilova, 1980; Ménard et al., 2000; http://www.Fishbase.org, accessed 01-10-2018). Rainbow runner, Spanish mackerel, and several fish species in the family Lutjanidae such as red emperor snapper also figure prominently in the local fish markets around Lae (Coffey, 2018c). These species are caught by the recreational and artisanal fisheries closer to shore but mostly to the south of Lae, several nautical miles from the DSTP area.

Most of the fish species people consume from the Huon Gulf are caught well outside the DSTP project area, as explained above. This is because of (a) the lack of clear water coastal reefs, offshore reefs and seamounts, which normally sustain a great variety of fish communities, including those species people consume from the Huon Gulf, and (b) the large daily input of sediment transported downstream by the Markham River as well as the Busu, Bupu and other nearby rivers (Figure 2-4), which cover much of the seafloor in the Project area (Coffey, 2018c).

Top trophic level fish that people consume (e.g., various tuna species) have large home ranges and a varied diet (Coffey, 2018c; http://www.Fishbase.org) and therefore, are expected to be minimally exposed to metals from subsurface DSTP plumes in the Huon Gulf. Fish species typically observed on slopes to around 500m are mostly absent in the DSTP study area (replaced by the dominance of dwarf gulper sharks, that are not reported to be consumed by people). Although fishing takes place around the DSTP outfall from the shoreline, the fish caught and consumed by people in this area comprises species such

as saddletail snapper or mangrove jack. While more local in their home range than tuna (Coffey, 2018c; http://www.Fishbase.org), these species do not typically inhabit deep waters or the mid-water column, being bottom dwelling fish found in waters approximately 100m deep or less, and therefore shallower than the DSTP outfall. These species, therefore, are also unlikely to have direct or indirect contact with DSTP subsurface plumes. Notwithstanding the DSTP area not being a targeted deep sea fishing location (with fishing occurring only in waters shallower than the DSTP outfall depth), it is conservatively assumed in this assessment that saddletail snapper and mangrove jack and the species caught further to the south of Lae (e.g., mahi mahi, red emperor, rainbow runner, sharptooth jobfish, bigeye trevally and pennantfish) and sold at the markets in Lae, could also occasionally occur in the DSTP area.

As discussed in Section 2.2, the bulk of the tailings (60%) is predicted to descend rapidly down the canyon slope as a bottom-attached density current and deposit on the seafloor (Figure 2-2). DSTP modelling results also predict that sediment plumes are likely to accumulate on the walls and seafloor of the Markham Canyon and, and not on the steep continental shelf. Visual observations of the seafloor indicate that the benthic habitats are characterised by fine, silty, easily re-suspended sediments, which are accumulated in deposits of unknown thickness (Coffey, 2018a). The seafloor is generally flat or gently sloping and lacks complex three dimensional structure such as rock, rubble, or aggregate reef habitat that would promote colonisation by benthos. Consistent with the very limited habitat available, the benthic fauna is generally low in density and diversity (Coffey, 2018a), consisting primarily of occasional unidentified species of shrimp, sea whips, and ophiuroids. The very sparse benthic community observed is due in part to periodic disturbance resulting from high sedimentation and seafloor mass movement events (IHAConsult, 2018a; Coffey, 2018a). As a result of the sparse and ephemeral nature of the benthic community, there is unlikely to be significant transfer of metals from benthic organisms to larger organisms in the Huon Gulf (Figure 2-8).

Furthermore, the disconnection of deep waters from upper layers of seawater where fish are collected for consumption reduces the likelihood of metals in seafloor sediments from becoming bioavailable and accumulating in fish consumed by people. This observation is supported by the types of trophic pathways present (as discussed above) and actual metal tissue data from the Huon Gulf, and from other active DSTP sites in the Asia-Pacific region (as discussed in Section 3.0). Thus, the transfer of metals from benthic sediments and/or benthic organisms to fish is not considered a complete pathway into fish consumed by people in the Huon Gulf. The important pathways to consider regarding metals in fish consumed by people are direct and indirect exposure to bioavailable metals in the DSTP subsurface plumes.

Based on information collected thus far in the Huon Gulf, biota that could occur in the vicinity of the DSTP discharge and subsurface plumes, such as zooplankton, micronekton, and certain species of pelagic fish, could be exposed to metals and form a complete pathway for metals in fish that people consume. Therefore, the remainder of this evaluation focuses on the DSTP subsurface plume pathways.

2.5. Summary of Viable Pathways of Metal Bioaccumulation from DSTP in Fish Consumed by People

Figure 2-8 is a conceptual diagram of the complete pathways by which metals from DSTP in the Huon Gulf could conceivably accumulate in fish that people consume based on the information presented in this chapter.

Site-specific modeling shows that the tailings is predicted to mix with the seawater and form a subsurface plume over depth range of 300-500m and at a distance of up to 2.7km from the DSTP outfall (Figure 2-3). Site-specific modeling further indicates that biota would be exposed to subsurface tailings plume dilutions between 1:1,000 and 1:1,800 at about 400m depth (Figure 2-3). Thus, biota in the Huon Gulf are predicted to be exposed to dilute concentrations of metals in the liquid fraction from a proposed DSTP discharge. According to studies conducted by CSIRO (2018), less than 20% of the total copper and nickel in tailings liquor are bioavailable as determined by dilute acid-soluble measurements of these metals. Zinc, however, appears to be in a bioavailable form primarily as evidenced by similar concentrations of total and acid-soluble zinc in tailings liquor[1]. These results suggest that metals such as copper and zinc may be released to the water column in a dissolved form within the subsurface plume, which could be bioavailable to plankton, micronekton, and fish exposed to the plume. The degree of metal uptake by different fauna will be species-specific and metal-specific and will depend on organism movement patterns, which will influence species exposure to the subsurface plumes and any dissolved metals present. Species uptake and depuration rates for a given metal will also be an important factor affecting organism bioaccumulation of metals.

Fish that people consume may accumulate metals originating from the subsurface plumes via direct uptake via the gills (bioconcentration from the water) as well as from prey that have accumulated metals from the subsurface plume (dietary uptake). The degree of bioconcentration and bioaccumulation will be a function of the metal chemical properties (e.g., ionic form and solubility) and the diet of the fish species, as well as that of its prey items. In addition, there is extensive research documenting that fish species and age-specific differences in metal uptake, metabolism, and depuration of metals are also important factors affecting tissue concentrations observed (USEPA 2007).

[1] The tailings liquor is the dissolved fraction of the tailings slurry with all solids removed after filtering through a 0.45µm membrane.

Figure 2.7: Food web diagram for some of the commonly consumed fish species in Huon Gulf. Fish species noted in green are consumed by people in PNG. Red arrows represent potential direct pathways between bottom-feeding fish and fish consumed by people.

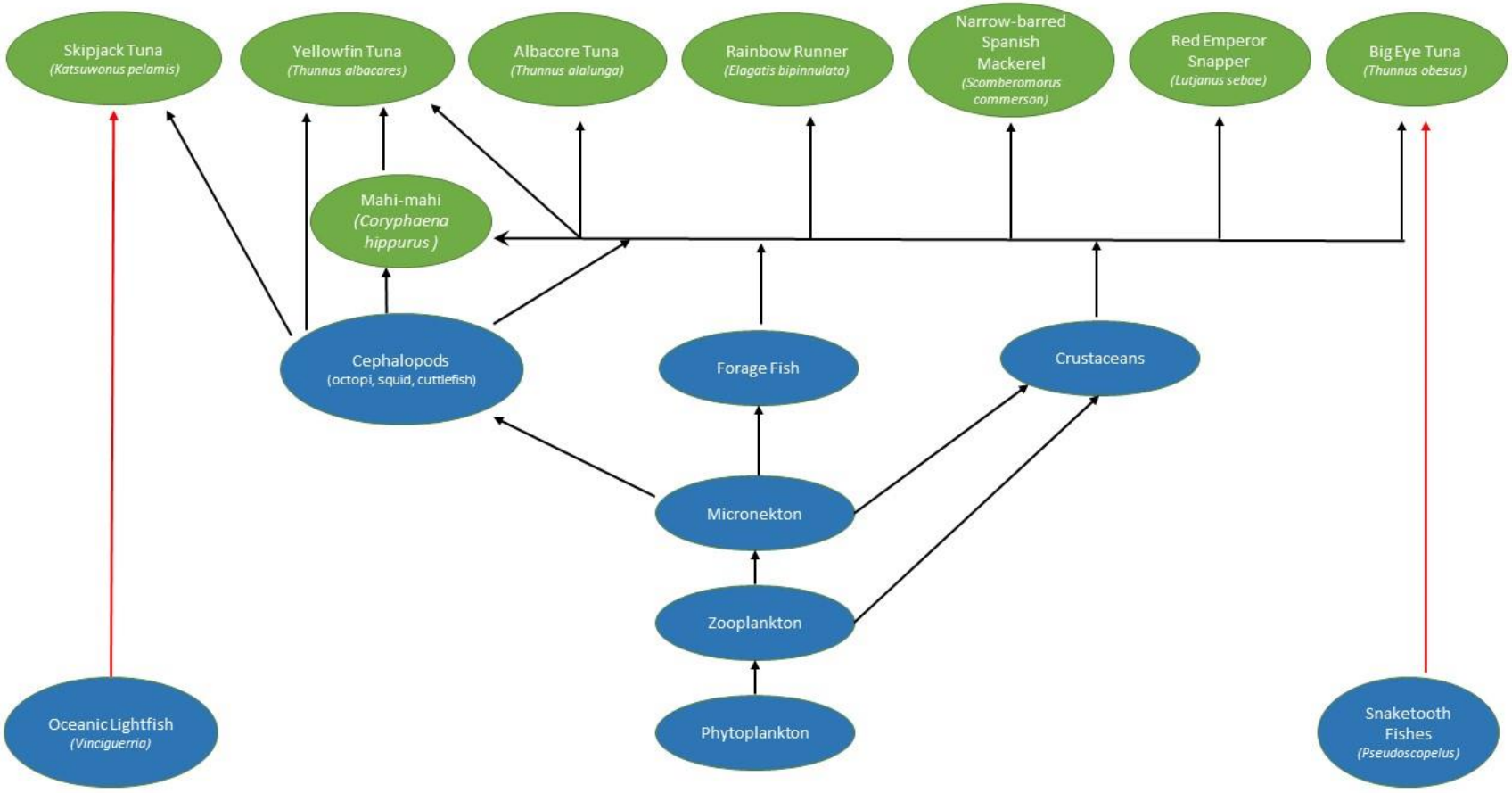
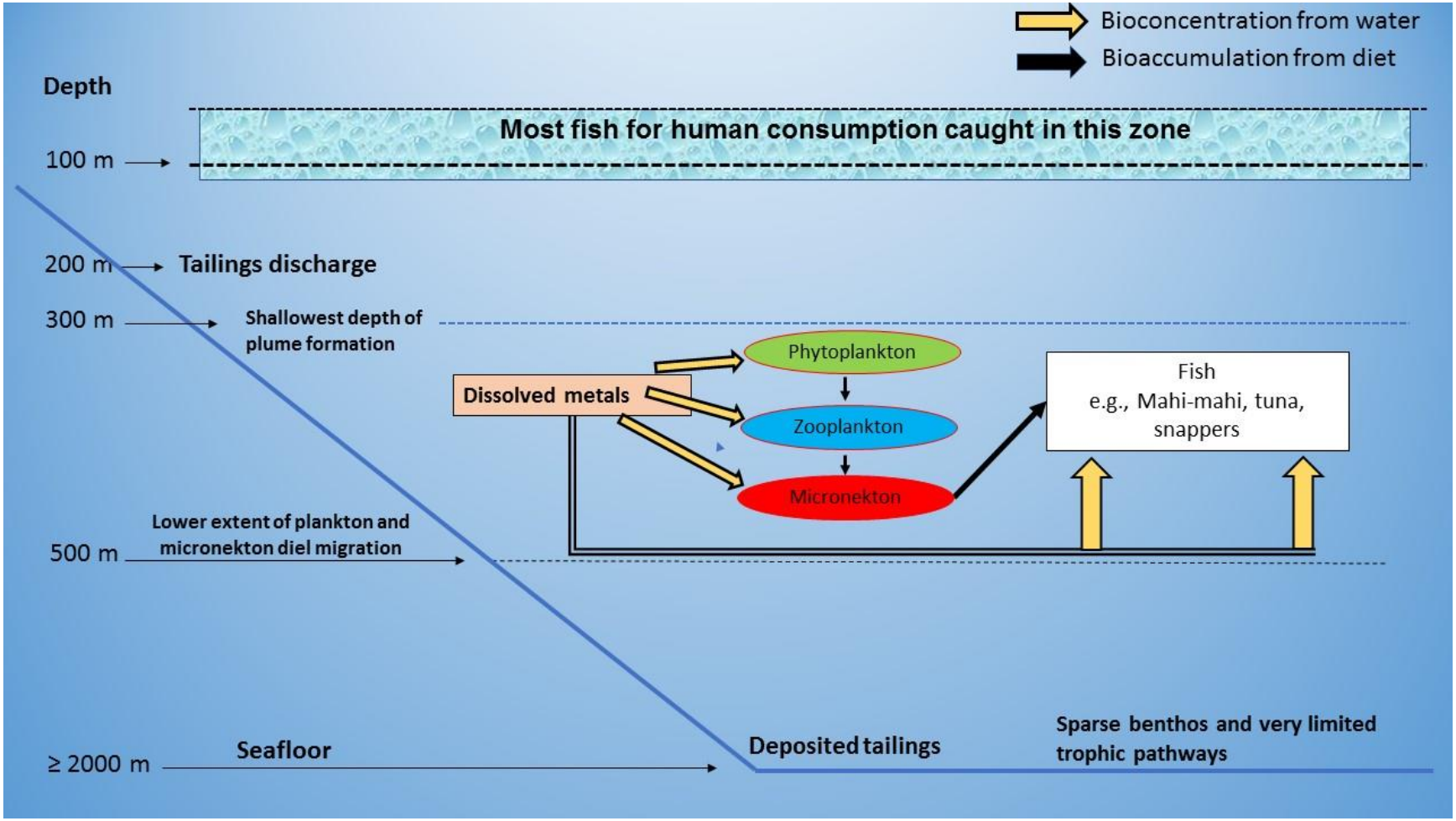


Figure 2.8: Conceptual diagram depicting the major pathways by which metals from a DSTP discharge could potentially be accumulated in fish consumed by people in the proposed project area in the Huon gulf. Note: depths are not to scale.



3. Observed Bioaccumulation of Metals from DSTP in Fish Consumed by People

Several studies have been conducted characterising metal concentrations in tissues of zooplankton, micronekton, and various fish species, including those that people consume in the Huon Gulf and elsewhere in the Asia-Pacific region. Table 3-1 summarises maximum metal concentrations observed in fish that people consume in the Huon Gulf (Coffey, 2018d). Appendix B contains a summary of metal data from the tissue of zooplankton and micronekton (Coffey, 2018b) and for pelagic fish (Coffey, 2018d).

3.1. Information from the Huon Gulf and DSTP Sites in the Region

Data from the Huon Gulf indicate that the food safety standards for arsenic and mercury are currently exceeded in some fish consumed by people from the Huon Gulf in the absence of a DSTP discharge (Coffey, 2018d). Other metals examined such as copper, nickel, zinc, and manganese occur at low concentrations in fish tissue and infrequently exceed food safety guidelines (there are no FSANZ food standards for nickel or manganese). This information suggests that food safety standards may be exceeded in fish for arsenic and mercury due to natural and human sources. Natural sources include discharges from major rivers in the region (e.g., Markham River) that contribute large amounts of sediment and associated metals into the Huon Gulf, derived from natural geological and human sources in PNG (Coffey, 2018a). Human sources of metals may include wastewater discharges, runoff from the city of Lae and perhaps shipping activities. The importance of natural sources of elevated arsenic and mercury concentrations in some fish species has been demonstrated by fish tissue data collected from other sites in PNG such as Lihir and Misima (CSIRO, 2005; Brewer et al., 2007, 2012), in which elevated mercury and arsenic concentrations were observed prior to mining (Tables 3-2 through 3-6).

Tissue data from different trophic levels (excluding shark species, which are not being consumed by people) within the Huon Gulf indicates that, with the possible exception of arsenic and mercury, metals evaluated in this study do not biomagnify in fish consumed by people (such as the blackspotted croaker (*Protonibea diacanthus*), mangrove jack (*Lutjanus argentimaculatu*s), saddletail snapper (*Lutjanus malabaricus*), sharptooth jobfish (*Pristipomoides typus*), bigeye trevally (*Caranx sexfasciatus*), and Pennantfish (*Alectis ciliaris*) (Table 3-7). These data are visualised in Figure 3-1 and include data for fish muscle tissue only, because the muscle is the main part of the fish that is consumed by people. Liver concentrations of metals were also measured in several fish species sampled from the Lae fish market to provide information on metal burden in fish (Coffey, 2018d). In general, liver concentrations of arsenic, copper, and mercury were higher than muscle concentrations in samples of several fish species that people consume, however, sample size was not sufficient to evaluate these data statistically (Coffey, 2018d). Notwithstanding the small number of fish analysed, results of tissue analyses suggested that arsenic was 1 to 2 times higher, copper was 2 to 12 times higher, mercury was 1 to 2 times higher, and zinc was 10 to 17 times higher in liver than in muscle (Coffey, 2018d). Higher concentrations for these metals in liver were also reported in fish species people consume in Misima and Lihir during pre-mining EIS studies (Coffey, 2018d). Other metals of concern in this analysis (nickel and manganese) occurred at very low concentrations in both muscle and liver (Coffey, 2018d). Data for these metals were not available for Lihir or Misima (Coffey, 2018d). Arsenic, mercury, copper, and zinc are often accumulated in the liver of fish (as well as other vertebrates), which removes these metals from the blood. The liver contains many proteins (e.g., metallothionine) that bind and/or detoxify these metals into non-toxic forms that are typically excreted unless exposure concentration and duration exceed transformation rates in the liver (or kidney in the case of mercury; Klassen et al. 1996). Transformation rates of these metals is very rapid (Klassen et al., 1996) and therefore, toxic accumulations are highly unlikely in fish people consume.

Available information shows that for many of the metals examined, tissue metal concentrations are highest in zooplankton and micronekton in the Huon Gulf. Similar findings have been reported for DSTP sites in PNG (Brewer et al., 2007 and 2012; CSIRO, 2005) and in Indonesia (Angel et al., 2013), as well as studies at the jarosite barge disposal site southeast of Tasmania (CSIRO 1994). In the latter case, from 1991 to 1994, CSIRO conducted food chain tissue analyses related to dumping of jarosite, the residue from the zinc-smelting process that contained elevated levels of zinc, cadmium, lead, arsenic and mercury. That study also included integrated modeling of metal uptake through the food chain from micronekton to southern bluefin tuna. The study found that heavy metal bioaccumulation in aquatic biota was low, including in southern bluefin tuna, even after 24 years of ocean surface disposal operations. An important distinction in the jarosite study, compared with DSTP examples, is that exposure of pelagic fish to metals was throughout the surface waters and not dependent only on vertically migrating organisms.

In addition to the jarosite study, data reported in Brewer et al. (2012) for the Lihir DSTP site are especially relevant to the present analyses and includes the metal concentrations reported for different trophic levels at reference sites for the Lihir study. From baitfish to pelagic fish, all metals examined diminished in concentration at both the mine and reference sites, except for mercury, which increased in concentration with organism trophic level (i.e., biomagnified) at the reference site. At the higher trophic level (baitfish to pelagic fish), biomagnification factors (BMFs) were similar for most metals and generally less than 1.0, indicating that most of the metals of concern did not increase in concentration with increasing trophic level (Brewer et al., [2012]). These data indicate that biomagnification of metals in fish consumed by people has not been observed from the research and monitoring studies at the DSTP operations in PNG or Indonesia discussed above.

Based on the information collected for the Huon Gulf and DSTP sites in the Asia-Pacific region, metal concentrations in fish tissue are generally below the joint Food Standards Code for Australia and New Zealand (FSANZ, 2011). Furthermore, with the exception of shark species, there appears to be no evidence for metal biomagnification at these sites as shown by much lower concentrations in higher trophic level fish than in lower trophic levels. This conclusion is further supported based on published information related to species metal uptake and bioaccumulation potential for the metals of concern (see Section 3-2).

Table 3-1: Maximum concentrations of total metals (mg/kg, wet weight) measured in muscle tissue of fish consumed by people from the Huon Gulf in comparison with Australian and New Zealand food safety standards (FSANZ, 2011). Tissue concentrations were derived from either fish collections or from market fish in PNG (Coffey, 2018b).

Common name	Species	Copper (mg/kg)	Zinc (mg/kg)	Arsenic (mg/kg)	Mercury (mg/kg)	Nickel (mg/kg)	Manganese (mg/kg)
Blackspotted croaker	*Protonibea diacanthus*	0.13	2.2	0.96	0.26	<0.06	<0.1
Mangrove jack	*Lutjanus argentimaculatus*	0.13	3.10	**2.6**	**0.53**	<0.06	<0.1
Saddletail snapper	*Lutjanus malabaricus*	0.11	3.40	**6.2**	0.36	<0.06	<0.1
Sharptooth jobfish	*Pristipomoides typus*	0.14	2.50	1.80	0.35	<0.06	<0.1
Bigeye trevally	*Caranx sexfaciatus*	0.50	4.80	0.60	**0.71**	<0.06	0.11
Pennantfish	*Alectis ciliaris*	0.19	3.10	**5.0**	0.25	<0.06	<0.1
Common pike eel	*Muraenesox bagio*	0.10	2.60	**32**	0.24	<0.06	0.12
FSANZ Standard		2*	15*	2	0.5	NGA	NGA

NGA = no guideline available

FSANZ (2011) exceedances shown in **bold**

* denotes from FSANZ (2011). Generally Expected Levels (GELS) for Metal Contaminants - Additional guidelines to Max levels in Standard 1.4.1 - Contaminants and Natural Toxicants. The guidelines are given for median and 90th percentile values however only the 90th percentile value is presented on the basis the guidelines recommend that exceedance of the 90th percentile value should initiate further investigation into the source of the concentration.

Table 3-2: Maximum concentrations of arsenic (mg/kg, wet weight) in muscle tissue of fish species in the family Lutjanidae reported in Coffey (2018b) for pre-mining EIS characterisation studies at Woodlark, Misima and Lihir.

Species	Woodlark (mg/kg)	Misima (mg/kg)	Lihir (mg/kg)	FSANZ standard (mg/kg)
Oblique-banded snapper *Pristipomoides zonatu*	16	-	13.86	2
Saddle-back snapper *Paracaesio kusakarii*	3.40	4.5	2.83	2
Small-toothed jobfish *Aphareus rutilans*	7.10	5.18	10.28	2
Banded snapper *Pristipomoides sp.*	4.70	8.94	7.43	2
Red bass *Lutjanus bohar*	15	-	-	2
Yellowlined snapper *Lutjanus rufolineatus*	2.5	-	-	2

Table 3-3: Maximum concentrations of copper (mg/kg, wet weight) in muscle tissue of fish species in the family Lutjanidae reported in Coffey (2018b) for pre-mining EIS characterisation studies at Woodlark, Misima and Lihir.

Species	Woodlark (mg/kg)	Misima (mg/kg)	Lihir (mg/kg)	FSANZ standard (mg/kg)
Oblique-banded snapper *Pristipomoides zonatu*	0.20	-	**2.37**	2
Saddle-back snapper *Paracaesio kusakarii*	0.09	0.56	**2.75**	2
Small-toothed jobfish *Aphareus rutilans*	0.21	0.62	1.58	2
Banded snapper *Pristipomoides sp*	0.59	0.20	1.25	2
Red bass *Lutjanus bohar*	0.18	-	-	2
Yellowlined snapper *Lutjanus rufolineatus*	0.20	-	-	2

Table 3-4: Maximum concentrations of mercury (mg/kg, wet weight) in muscle tissue of fish species in the family Lutjanidae reported in Coffey (2018b) for pre-mining EIS characterisation studies at Woodlark, Misima and Lihir.

Species	Woodlark (mg/kg)	Misima (mg/kg)	Lihir (mg/kg)	FSANZ standard (mg/kg)
Oblique-banded snapper *Pristipomoides zonatu*	0.20	-	<0.1	0.5
Saddle-back snapper *Paracaesio kusakarii*	0.04	<0.1	0.16	0.5
Small-toothed jobfish *Aphareus rutilans*	0.03	0.12	<0.01	0.5
Banded snapper *Pristipomoides sp*	**0.52**	**0.70**	**1.40**	0.5
Red bass *Lutjanus bohar*	0.4	-	-	0.5
Yellowlined snapper *Lutjanus rufolineatus*	0.17	-	-	0.5

Table 3-5: Maximum concentrations of nickel (mg/kg, wet weight) in muscle tissue of fish species in the family Lutjanidae reported in Coffey (2018b) for pre-mining EIS characterisation studies at Woodlark, Misima and Lihir. There is no FSANZ standard for nickel.

Species	Woodlark (mg/kg)	Misima (mg/kg)	Lihir (mg/kg)
Oblique-banded snapper *Pristipomoides zonatu*	<0.06	-	<0.06
Saddle-back snapper *Paracaesio kusakarii*	<0.06	-	<0.06
Small-toothed jobfish *Aphareus rutilans*	<0.06	-	<0.06
Banded snapper *Pristipomoides sp.*	<0.06	-	<0.06
Red bass *Lutjanus bohar*	<0.06	-	-
Yellowlined snapper *Lutjanus rufolineatus*	<0.06	-	-

Table 3-6: Maximum concentrations of zinc (mg/kg wet weight) in muscle tissue of fish species in the family Lutjanidae reported in Coffey (2018b) for pre-mining EIS characterisation studies at Woodlark, Misima, and Lihir.

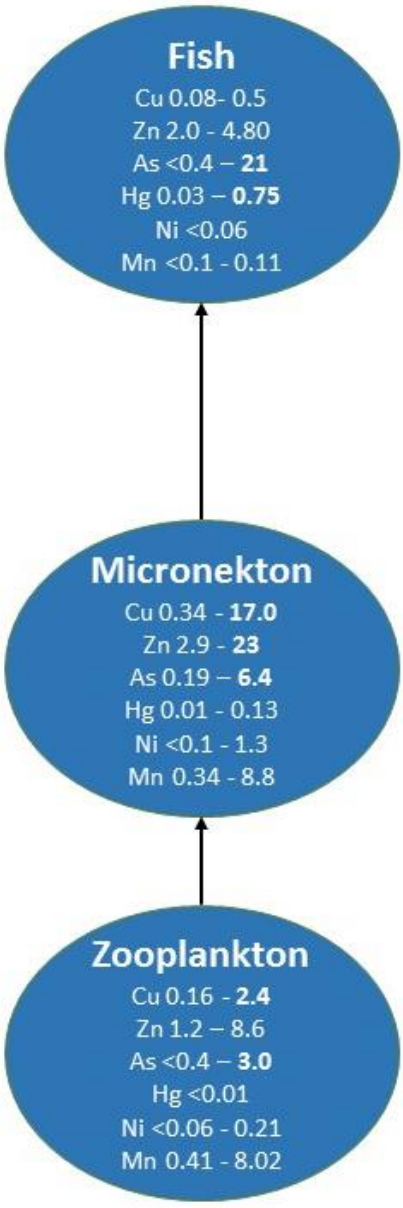
Species	Woodlark (mg/kg)	Misima (mg/kg)	Lihir (mg/kg)	FSANZ standard (mg/kg)
Oblique-banded snapper *Pristipomoides zonatu*	5.10	-	3.24	15
Saddle-back snapper *Paracaesio kusakarii*	3.50	6.76	3.24	15
Small-toothed jobfish *Aphareus rutilans*	3.40	8.36	5.12	15
Banded snapper *Pristipomoides sp.*	6.5	-	-	15
Red bass *Lutjanus bohar*	3.9	-	-	15
Yellowlined snapper *Lutjanus rufolineatus*	3.7	-	-	15

Table 3-7: Concentrations of total metals (mg/kg, wet weight) in fish (including shark species), micronekton, and zooplankton species collected at various depths in the Huon Gulf, PNG (from Coffey, 2018b; Coffey 2018d).

Tissue type	Species	Depth (m)	Copper (mg/kg)	Zinc (mg/kg)	Arsenic (mg/kg)	Mercury (mg/kg)	Nickel (mg/kg)	Manganese (mg/kg)
Fish	Dwarf gulper shark	100-600	0.07 - 0.18	1.90 - 3.70	6.9 - 36	0.02 - 2.20	<0.06 - 0.07	<0.1 - 0.18
	Longfin gulper shark	300-500	0.09 - 0.25	2.10 - 2.70	12 - 38	0.35 - 1.30	<0.06 - <0.06	<0.1 - 0.11
	Gulper shark	500-600	0.11	3.30	33	1.50	<0.06	<0.1
	Blackspotted croaker	200-300	0.13	2.2	0.96	0.26	<0.06	<0.1
	Mangrove jack	0-200	0.11 - 0.13	2.60 - 3.10	<0.4 - 2.6	0.03 - 0.53	<0.06	<0.1
	Saddletail snapper	30-200	0.08 - 0.11	2.20 - 3.40	2.3 - 6.2	0.05 - 0.36	<0.06 - <0.06	<0.1 - 0.10
	Sharptooth jobfish	30-200	0.11 - 0.14	2.40 - 2.50	0.83 - 1.80	0.05 - 0.35	<0.06 - <0.06	<0.1 - <0.1
	Bigeye trevally	0-200	0.29 - 0.50	3.40 - 4.80	<0.4 - 0.60	0.34 - 0.71	<0.06 - <0.06	<0.1 - 0.11
	Pennantfish	30-40	0.19	3.10	5.0	0.25	<0.06	<0.1
	Fatspine spurdog	100-300	0.11 - 0.16	2.0 - 2.50	5.5 - 21	0.08 - 0.75	<0.06 - <0.06	<0.1 - <0.1
	Common pike eel	100-200	0.10	2.60	32	0.24	<0.06	0.12
Micronekton	Decapoda A	0-500	1.7	7.3	0.19	0.02	0.54	0.83
	Decapoda B	0-250	5.8	11	0.43	0.02	<0.1	1.1
	Gonostomatid A	0-250	2.2 - 4.8	10-18	0.29 - 0.64	0.01 - 0.01	0.24 - 1.3	1.7 - 2.1

Tissue type	Species	Depth (m)	Copper (mg/kg)	Zinc (mg/kg)	Arsenic (mg/kg)	Mercury (mg/kg)	Nickel (mg/kg)	Manganese (mg/kg)
	Gonostomatid B	0-250	3.4	13	0.9	0.01	0.36	3.2
	Pandalid	0-500	17.0	8.1 – 9.1	0.65 - 1.0	0.03	<0.1 - 0.16	1.5 - 2.0
	Anguilliform	0-250	1.4 - 3.0	6.2 - 8.5	0.26 - 0.45	0.02 - 0.02	0.14 - 0.14	1.3 - 2.7
	Trachichthyid	0-250	2.5	8.8	0.52	0.03	0.25	2.7
	Bregmaceros sp.	0-500	1.4 - 4.0	11-16	0.33 - 0.36	0.02 - 0.03	0.25 - 1.1	3.0 - 3.2
	Chauliodus sloani	0-500	0.34 - 0.46	2.9 - 4.6	0.5 - 0.59	0.02 - 0.02	<0.1 - <0.1	0.65 - 0.94
	Conger eel	0-500	1.8	6.6	0.44	0.13	<0.1	0.34
	Mysidacea	0-500	4.8	12	0.24	0.01	0.14	1.6
	Pteropoda	0-500	3.8	7.2	0.37	0.1	0.97	8.8
	Xenodermichthys nodulosus	0-500	1.4 - 2.9	19 - 23	5.0 - 6.4	0.03- 0.04	0.13 - 0.18	1.6 - 2.3
Zooplankton	Bulk zooplankton sample	0-100	0.6 - 2.3	2.6 - 7.1	0.82 - 3.0	<0.01	0.1- 0.21	0.97 - 8.02
	Bulk zooplankton sample	0-250	0.42 - 2.4	1.7 - 8.6	0.47 - 1.9	<0.01	0.08 - 0.17	0.56 - 1.95
	Bulk zooplankton sample	0-500	0.16 - 0.58	1.2 - 4.2	<0.4 - 1.6	<0.01	<0.06 - 0.17	0.41 - 3.23

Figure 3-1: Tissue metal concentrations (mg/kg wet weight) observed in three trophic levels for samples 0-500m depth (lowest trophic level at the bottom) of the Huon Gulf, PNG. Cu = copper, Zn = zinc, As = arsenic, Hg = mercury, Ni = nickel, Mn = manganese. Fish data are based on species people consume and do not include shark species. (Sources of data: fish – Coffey 2018d; micronekton and zooplankton – Coffey 2018b). Bold values represent tissue concentrations that exceed FSANZ standards.



3.2. Metal Bioaccumulation Information from the Literature

Many published sources report generally low bioaccumulation and limited or no biomagnification of most of the metals of interest in the Huon Gulf (Brewer et al 2012; Neff 2002), consistent with the field information presented in the previous section. Metals frequently occur as charged ions in marine systems and require active transport to facilitate uptake by invertebrates and fish (Drexler et al., 2003; Neff, 2002). Active transport in this case implies that the uptake rate of metals declines as the concentration of metals in water increases. This is especially true for the essential metals (copper, nickel, zinc, manganese, and arsenic; Neff, 2002) that are required for proper biological functioning at low concentrations (Drexler et al 2003). Thus, at low concentrations in water, marine biota will actively take up essential metals to satisfy their biological requirements and at higher concentrations, marine biota will actively exclude and/or excrete these metals.

Brewer et al (2012) reported that the metals of interest in this study, i.e., arsenic, copper, nickel, inorganic mercury, manganese, and zinc, have generally low bioaccumulation factors in higher trophic levels such as fish and that these metals do not biomagnify in marine food webs. Others, in a meta-analysis of published findings, for example Cardwell et al. (2013) concluded that copper, nickel, zinc, and several other metals generally do not biomagnify. Wang (2002) attributes the lack of biomagnification of metals between levels in the 'classic' pelagic food chain (phytoplankton to zooplankton to nekton) to the effective depuration of metals by some zooplankton (e.g., copepods) and the low assimilation efficiency of metals by marine fish.

The following summarises relevant literature regarding bioaccumulation and biomagnification potential for each metal of interest in different trophic levels in marine systems. Tropical marine systems are highlighted where information is available.

Arsenic

Most marine animals, including fish, exhibit little or no bioaccumulation of inorganic arsenic (Neff 2002). However, total arsenic (mostly in the form of organoarsenic compounds) is frequently observed in marine biota, indicating that organic forms of arsenic are accumulated in fish, probably through the diet (trophic transfer; Neff 2002). As described in Suedel et al. (1994), there is some potential for arsenic to biomagnify in marine food webs in higher trophic levels (e.g., sharks) based on a study on the western coast of Canada published by LeBlanc and Jackson (1973). However, in a more recent field bioaccumulation factor (BAF) study (Hong et al., 2014), it was determined that arsenic did not appear to biomagnify in either freshwater or marine food webs. Mean arsenic BAF values for marine fish, bivalve, crab, and shrimp were low (200L/kg, 316L/kg, 398L/kg, and 158L/kg, respectively, based on the log BAF values from Hong et al. 2014) as compared to BAFs commonly reported for organic chemicals (BAFs > 5000). In addition, Athalye et al. (2001) conducted a field BAF study in which predator-prey relationships were evaluated in Thane Creek, India (a system with mean salinity of 22.68ppt). The authors found that arsenic bioaccumulated in phytoplankton, with a BAF of 50.95L/kg; however, arsenic was not detected in the corresponding grazing flathead grey mullet (*Mugil cephalus*) (Trophic Level [TL] 3), indicating that arsenic biomagnification did not occur in this food chain. The U.S. Environmental Protection Agency recommends a default bioconcentration factor (BCF) of 114 L/kg (applied to marine and freshwater fish on wet weight basis) based on several peer reviewed studies (USEPA 1999).

Copper

Athalye et al. (2001) found that copper BAFs were higher in lower (detrital) trophic levels of the food chain in comparison to higher (grazing) trophic levels in an estuarine creek in India. The phytoplankton (TL1) copper BAF was 235.84L/kg (dry weight) and the corresponding grazing flathead grey mullet (*Mugil cephalus*) (TL3) BAF was 10.18L/kg (dry weight). In addition, the polychaete (TL2) copper BAF was

371.43L/kg and the corresponding grazing long whiskers catfish (*Mystus gulio*) (TL3) had a BAF of 29.5L/kg. These data demonstrate relatively low bioaccumulation potential of copper in marine organisms as shown by the relatively low BAFs. The Athalye et al. (2001) study also strongly indicates that copper did not biomagnify in this food chain.

There is little evidence that copper biomagnifies at higher trophic levels in marine environments. Suedel (1994) reviewed several studies and found no indication of biomagnification of copper in marine systems. Cardwell et al. (2013) noted that copper generally does not biomagnify in food chains made up of primary producers, macroinvertebrate consumers, and fish; however, there are some special situations where biomagnification of copper appears to occur, such as marine food chains consisting of bivalves, herbivorous gastropods at TL2 and carnivorous gastropods at TL3. The U.S. Environmental Protection Agency recommends a default BCF of 710L/kg (applied to both marine and freshwater fish on wet weight basis) based on several peer reviewed studies (USEPA, 1999).

Nickel

Nickel has been shown to bioaccumulate at low levels (BAFs generally less than 500) in lower trophic level marine organisms (phytoplankton and zooplankton), and have little or no bioaccumulation in higher trophic levels including fish (Brown and Luoma, 1995). Alhashemi et al. (2012) found no indication of biomagnification of nickel in an estuarine site in India and Cardwell et al. (2013) and Suedel et al. (1994) noted that nickel generally does not biomagnify in food chains made up of primary producers, macroinvertebrate consumers, and fish. Drexler et al. (2003) report that biomagnification of nickel along the aquatic food chain, including marine food chains, does not occur. The U.S. Environmental Protection Agency recommends a default BCF of 78L/kg (applied to both marine and freshwater fish on wet weight basis) based on several peer reviewed studies (USEPA, 1999).

Manganese

Most aquatic species, including tropical marine fish species, actively transport and utilize relatively high concentrations of manganese as compared to the other metals examined in this study due to its significant nutritional requirement (WHO, 2004). Thus, manganese has been shown to bioconcentrate in marine organisms however, the degree of bioconcentration decreases with increasing trophic level. For example, bioconcentration factors reported by WHO (2004) and the U.S. Agency for Toxic Substances and Disease Registry (ATSDR, 2008) are higher at lower trophic levels (300-5,500L/kg for marine algae) and much lower for intertidal mussels (800-830L/kg), and even lower in fish (35-930L/kg). BAFs for manganese are also reported to be low in marine fish. Alhashemi et al. (2012) reported fish manganese BAFs of 45 to 224L/kg (dry weight) in *Silurius trisostegus* (TL4) and *Barbus luteus* (TL4), respectively. Because manganese is highly metabolised by marine biota, including fish (WHO 2004), manganese does not biomagnify in fish. This conclusion has been supported by other research as well (Suedel et al., 1994; Neff 2002; WHO 2004). The USEPA does not have recommended default BCF for fish because few studies have rigorously examined this metal (USEPA, 1999).

Mercury

Bioaccumulation of inorganic mercury is generally very low in marine organisms (BAFs < 1000L/kg) and biomagnification of inorganic mercury is very rare in marine systems (Neff, 2004). Inorganic mercury, even if complexed with organic matter, is not bioavailable to marine fauna (Neff, 2002) as indicated by its relatively low toxicity to marine life (USEPA, 1996). However, there is extensive evidence indicating that methylated mercury does bioaccumulate and biomagnify in marine food webs and, because of its widespread distribution via atmospheric deposition, mercury is a global concern (see Minamata Convention on mercury:
http://www.mercuryconvention.org/Portals/11/documents/conventionText/Minamata%20Convention%20o

n%20Mercury_e.pdf.). Biological methylation of inorganic mercury by sediment anaerobic bacteria was the likely cause of the Minamata disease in Japan and has since been implicated in many fish and wildlife population declines (Scheuhammer et al 2007). Methyl mercury is readily accumulated in aquatic organisms via diet and water ingestion and can be transferred to upper levels of the food chain resulting in high tissue concentrations and toxic effects. For example, Philip et al. (2003) reported BAFs ranging from 12,000 to 77,000L/kg (dry weight) in benthic invertebrates in Dickerson Bay and Appalachee Bay, Florida, in which methylmercury was the dominant form of mercury. Suedel et al (1994) and USEPA (1996) reviewed several marine laboratory studies, which indicated that methylmercury has the potential to biomagnify in marine food webs. The relative rates of mercury methylation and demethylation in the aquatic system have a greater effect on how much mercury is accumulated in aquatic organisms compared to the concentration of total mercury that is present. The USEPA recommends a default BCF of 3,530L/kg (applied to marine and freshwater fish on wet weight basis) based on inorganic mercury examined in peer reviewed studies (USEPA, 1999).

Zinc

Zinc has been shown to bioaccumulate at low levels primarily in marine invertebrates and not upper trophic level organisms such as fish (Neff 2004). Athalye et al. (2001) conducted a field BAF study in which predator-prey relationships were evaluated in Thane Creek, India, which has a salinity ranging up to 43ppt and a mean salinity of 22.68ppt. The authors found that zinc BAFs were higher in lower (detrital) trophic levels of the food chain in comparison to higher (grazing) trophic levels and that the fish BAFs were relatively low based on bioaccumulation thresholds used by many countries to flag chemicals for human health risk (e.g., USEPA 1999). The phytoplankton (TL1) zinc BAF was 636.3L/kg (dry weight) and the corresponding grazing flathead grey mullet (*Mugil cephalus*) (TL3) BAF was 89.45L/kg (dry weight). In addition, the polychaete (TL2) zinc BAF was 940.92L/kg and the corresponding grazing long whiskers catfish (*Mystus gulio*) [TL3] had a BAF of 130.97L/kg. This study strongly indicated that zinc did not biomagnify in this food chain.

Suedel et al. (1994) and Cardwell et al. (2013) reported that for marine fish, zinc does not biomagnify up the food chain as evidenced by trophic transfer factors slightly greater than 1.0 (between 1 and 2; a trophic transfer factor = 1.0 indicates that higher trophic level organisms have similar tissue concentrations of the metal as lower trophic levels). However, in several of the studies evaluated by Cardwell et al. (2013), it appeared that the slight biomagnification reported may have been because of a dietary deficiency that the fish needed to fill. Like arsenic, copper, nickel, and manganese, zinc is a nutritional requirement, and the fish in the respective studies may have been zinc-deficient due to low ambient zinc concentrations. The USEPA recommends a default BCF of 2,059L/kg (applied to marine and freshwater fish on wet weight basis) based on several peer reviewed studies (USEPA 1999).

The information summarised above indicates that, except for methylmercury, the metals of interest are unlikely to biomagnify in upper trophic level fish in marine systems such as the Huon Gulf. This conclusion supports the results derived from site-specific data in the Huon Gulf and other sites in the region.

4. Predicted Huon Gulf Metal Concentrations in Fish Consumed by People and Comparison with Food Safety Guidelines

4.1. Methodology for Predicting Metal Concentrations in Fish

The USEPA (1999) developed guidance for conducting ecological risk assessments which includes a screening methodology for calculating the concentration of a given contaminant in biota based on dietary uptake. Relevant excerpts from this method are provided in Appendix D.

The food chain model and associated bioaccumulation methodology used to calculate fish tissue concentrations of metals in this study were developed by the USEPA in conjunction with a rigorous peer review process that involved multiple federal and state environmental agencies, university scientists, and environmental conservation organisations. The USEPA's food chain model is the standard procedure used by all federal and state regulatory agencies in the U.S., Canada, and other countries to predict chemical tissue concentrations in various aquatic species at potential contamination sites. This modelling procedure is the standard method used by USEPA and other agencies to calculate predicted tissue concentrations in organisms at all trophic levels given either measured or predicted water concentrations of chemicals of concern. The food chain model procedure used in this study is also the standard protocol used by USEPA and other agencies in the U.S. to determine whether wildlife or humans are at potential risk from consuming contaminants of concern in fish and other aquatic life and is a critical component of ecological and human health risk assessments conducted in the U.S.

Based on the site conceptual model developed for the Wafi-Golpu Project (Section 2.5), the primary pathways by which fish consumed by people may accumulate metals is via direct exposure to the DSTP subsurface plume and associated liquid fraction of the tailings liquor, and via feeding on prey that have accumulated metals from direct or indirect exposure to the subsurface plumes (see Section 2.5). Direct exposure to the subsurface plumes is addressed by calculating the Bioconcentration Factor (BCF) between organism tissue concentration and the ambient seawater concentration where:

BCF = tissue concentration/seawater concentration

Exposure to metal via the diet is addressed by calculating the Trophic Transfer Factor (TTF) where:

TTF = tissue concentration in trophic level X / tissue concentration in trophic level X-1

Depending on the particular fish species under consideration, their diet will vary in terms of trophic levels comprising the prey items consumed as discussed in Section 2.4, and therefore the potential concentration of metal ingested. Other biological factors that will affect the predicted concentration of metal in fish species of interest are bioaccumulation rate and depuration rate of the metal.

For this screening methodology, conservative site-specific trophic level BCFs were calculated for each metal by dividing the maximum tissue concentration observed for a given trophic level species that people consume by the minimum dissolved metal water concentration reported. Water metal concentrations were derived from nearshore zone monitoring data (Coffey, 2018e), which may differ spatially with zooplankton, micronekton, and fish tissue concentrations used in this analysis. Dissolved concentrations for copper, mercury, and zinc were consistently below detection (below 1.0µg/L, 0.0001µg/L, and 5µg/L, respectively; Coffey, 2018e) in ambient nearshore marine waters. For these metals, BCFs were calculated using the detection limit as the water concentration, which is conservative because these metals (except mercury) are required elements for marine organism survival, growth, and reproduction (Neff, 2002). These metal concentrations (in µg/L) are: arsenic (1.3), copper (1.0), manganese (0.5),

nickel (0.5), zinc (5.0), and mercury (0.0001). As these metals concentrations were mostly below detection in the nearshore zone, it is considered that these concentrations are also representative of offshore water quality, which is not expected to have higher metals concentrations given the greater distance from Lae and terrestrial runoff. BCFs obtained through the above analyses are summarized in Table 4-1.

Conservative site-specific TTFs for each metal were calculated by dividing the tissue concentration in representative species in each trophic level by the tissue concentrations reported for representative species in the next lower trophic level (Table 4-1). Species depuration of metal was not incorporated (i.e., no loss of metal ingested via metabolism assumed) to provide a conservative estimate of tissue metal concentrations. Site-specific BCFs and TTFs are based on the water and tissue data, respectively, collected in the Huon Gulf to date. These data are not influenced by DSTP and are assumed to represent relevant bioaccumulation dynamics in the Huon Gulf.

Predicted concentrations of each metal of interest in the DSTP subsurface plume were estimated from 3-D modelling results assuming that organisms were exposed to the relatively concentrated part of the subsurface plume. The most concentrated region according to the model analysis represents a 1:1,800 dilution of the tailings discharge as explained in Section 2.2 (see Figure 2-3). The maximum dissolved metal concentration reported by CSIRO (2018) in the 1:1,000 dilution samples of tailing elutriates (see Section 2.2), was used in this analysis to yield a conservative estimate of the subsurface plume concentration for each metal to which biota would be exposed. Predicted metal concentrations used in these analyses are shown in Table 4-2.

For this analysis, zooplankton and micronekton were presumed to be constantly exposed to the predicted subsurface plume metal concentrations. Actual exposure of these fauna to the DSTP plume is likely to be much less than assumed in these calculations because of the large diel migrations of the plankton and micronekton observed in this system (Coffey, 2018b). Furthermore, the concentration of metals in the subsurface plumes will not be constant as assumed in these calculations, which also overestimates the likely exposure of these biota to metals in the DSTP discharge.

Metal concentrations in zooplankton were calculated assuming direct uptake from the plume using a BCF:

$$[Xmetal]_{zooplankton} = [Xmetal]_{plume} \times BCF_z$$

Where:

$[Xmetal]_{zooplankton}$ = the concentration of metal in the zooplankton

$[Xmetal]_{plume}$ = the concentration of metal in the DSTP subsurface plume

BCF_z = site-specific bioconcentration factor for uptake from surface water to zooplankton

Metal concentrations in micronekton (Trophic Level [TL] 2) were estimated by applying a site-specific TTF, to account for dietary ingestion, and a site-specific BCF for these biota (to account for bioconcentration), which were obtained from site water (Coffey, 2018e) and zooplankton and micronekton (Coffey, 2018b) concentrations for each metal obtained from the Huon Gulf. The equation used to calculate this estimated tissue concentration for micronekton is:

$$[Xmetal]_{micronekton} = ([Xmetal]_{zooplankton} \times TTF_m) + ([Xmetal]_{plume} \times BCF_m)$$

Where:

$[Xmetal]_{micronekton}$ = the concentration of metal in the micronekton

$[Xmetal]_{zooplankton}$ = the concentration of metal in the zooplankton

TTF_m = site-specific bioaccumulation factor for uptake from zooplankton to micronekton

$[Xmetal]_{plume}$ = the concentration of metal in the plume

BCF_m = site-specific bioconcentration factor for uptake from surface water to micronekton.

For fish that feed on micronekton (TL3 and TL4), fish tissue metal concentrations were calculated based on the TTF using the micronekton tissue concentration calculated above, and a site-specific bioconcentration factor for fish species to account for uptake from water. The TTF and BCF were based on an average of the maximum TTFs and BCFs derived from fish species that are consumed by people from the Huon Gulf. These species include: blackspotted croaker (*Protonibea diacanthus*), mangrove jack (*Lutjanus argentimaculatus*), saddletail snapper (*Lutjanus malabaricus*), sharptooth jobfish (*Pristipomoides typus*), bigeye trevally (*Caranx sexfasciatus*), and Pennantfish (*Alectis ciliaris*). The equation used to calculate predicted metal concentrations in fish consumed by people from the Huon Gulf is:

$$[X]TL3_{fish} = ([X]_{micronekton} * TTF_{TL3}) + ([X[metal]_{plume} * BCF_{fish})$$

Where:

$[X]_{fish}$ = the concentration of metal in fish species

$[X]_{micronekton}$ = the concentration of metal X in micronekton

TTF_{fish} = site-specific bioaccumulation factor for uptake from micronekton to fish

$[Xmetal]_{plume}$ = the concentration of metal in the plume

BCF_{fish} = site-specific bioconcentration factor for uptake from surface water to fish

The reliance on the BCF to calculate predicted metal concentrations in zooplankton is likely to be conservative because BCFs have generally been shown to be higher than dietary TTFs for zooplankton (USEPA, 1999). For higher trophic levels (micronekton, and fish) however, metal ingestion from prey may be as or more important than uptake from water (USEPA, 1999). Therefore, both BCF and TTFs need to be considered in a bioaccumulation factor (BAF) to predict metal tissue concentrations in each trophic level and particularly, for higher trophic level fish that people consume.

Total predicted fish metal concentrations using the above calculations are likely to overestimate actual fish tissue concentrations because the total tissue concentration assumes that fish will accumulate 100% of the metal from micronekton and 100% of the metal from water ingestion in the plume. Clearly, the fish tissue concentrations observed will be the result of some (unknown) combination of diet and water ingestion. For this screening, predicted tissue concentrations for micronekton and fish were evaluated based on BCFs and TTFs separately, which can be considered the boundaries of potential bioaccumulation in fish from the Huon Gulf consumed by people in the presence of DSTP. If the upper boundary of predicted tissue concentrations of metals is below food safety standards, there is high probability that metal concentrations will be at safe levels in fish consumed by people from the Huon Gulf.

Table 4-1: Summary of BCF and TTFs for each trophic level derived from the Huon Gulf data for each metal, which were used to estimate predicted fish tissue concentrations assuming a DSTP discharge. Fish BCFs and TTFs are based on data collected from the Huon Gulf for fish species people consume (see text). BCF = Bioconcentration Factor, TTF = Trophic Transfer Factor.

	Zooplankton BCF	Micronekton BCF	Fish BCF
Arsenic	2.3	0.77	2
Copper	2.4	17	0.13
Manganese	16.04	17.6	0.2
Nickel	0.42	2.6	0.12
Zinc	1.72	3.6	0.68
Mercury	100	1,300	5,300
	Zooplankton are assumed to ingest metals primarily from the water column. Therefore there is no TTF for zooplankton.	**Micronekton TTF**	**Fish TTF**
Arsenic		0.44	2.97
Copper		2.45	0.05
Manganese		0.56	0.02
Nickel		2.54	0.07
Zinc		1.68	0.29
Mercury		7.23	11.34

4.2. Predicted Fish Tissue Metal Concentrations

Table 4-2 summarises the predicted metal concentrations in representative fish species that people consume from the Huon Gulf based on BCFs only, and TTFs only. Appendix C contains the calculations used in this analysis.

Using the analytical process described in the previous section, fish that people consume from the Huon Gulf are unlikely to accumulate concentrations of metals that exceed food safety standards for zinc based on either water ingestion or diet only (Table 4-2). There are no FSANZ standards for manganese and nickel, and tissue concentrations are predicted to be low in fish species people consume. Arsenic and mercury were predicted to exceed food safety standards based on the conservative analysis; however, this is only because existing concentrations of these metals already exceed these standards. Copper is also predicted to exceed food safety standards in fish people consume based on the conservative analysis; however, this is because the pandalid shrimp had unusually high copper concentrations (Table 3-7; Coffey, 2018b). Crustacea such as pandalid shrimp that live in deep sea environments with low oxygen pressure typically use the copper-based protein haemocyanin for oxygen transport because the iron-based protein haemoglobin (common in most other invertebrates and vertebrates) is less efficient than hemocyanin in terms of oxygen transport in the organism (Strobel et al., 2012). If copper data for those species are removed from the micronekton copper data, the predicted fish concentration from ingestion of micronekton decreases from 3.0mg/kg (Table 4-2) to 0.20mg/kg, which is less than the FSANZ copper standard, and similar to existing concentrations of copper in fish that people consume (Table 4-2). In general, predicted tissue concentrations based on BCFs are lower when compared with predictions based on TTFs only. This is expected especially at higher trophic levels given the importance of the diet pathway in potential metal bioaccumulation.

Predicted concentrations of arsenic, nickel, mercury, manganese, and to a lesser extent, zinc, were similar to the observed range of tissue concentrations reported in fish species consumed from the Huon Gulf (Table 4-2). The higher predicted copper concentrations in this analysis as compared to observed fish tissue concentrations is related to the relatively high predicted copper concentrations (11µg/L) released from the tailings in the subsurface plume at 1,000 dilutions. This copper concentration at 1,000 dilutions was determined by the elutriate testwork by CSIRO (2018). DSTP modelling shows that 1,000 dilutions of the subsurface plumes are predicted to be achieved within about 1.3km from the outfall. As a result, micronekton is predicted to ingest a relatively high concentration of copper from zooplankton, which, in turn, may influence bioaccumulation of these metals in fish. However, as noted earlier, studies at Lihir have found no detectable bioaccumulation or biomagnification of metals in fish from lower trophic levels (Brewer, 2012). As noted previously, at least for copper and most of the other metals examined, depuration and elimination of metals from upper trophic level fish is well-known and therefore, concentrations are unlikely to be as high as predicted based on the conservative assumptions used in this analysis. Furthermore, the fish considered in these analyses infrequently inhabit the DSTP area because of habitat constraints as discussed in Section 2.4. Therefore, their exposure to subsurface DSTP plumes is expected to be much less than assumed. This assessment assumed continual contact with the maximum concentration of metals in subsurface plumes.

Typically, exposure assessments such as this study, consider a species home range, which is referred to as the area use factor (USEPA 1999). The area use factor is intended to account for the fact that a species may have a large home range and therefore, will likely only spend a part of their time exposed to the source of concern. In practice, the area use factor (expressed as a decimal from 0 to 1.0, where 1.0 indicates a very small home range and constant organism exposure) is multiplied by the concentration in the water or diet to yield a predicted exposure concentration that is then used in calculating tissue concentrations. In this study, most of the fish species that are consumed by people from the Huon Gulf are unlikely to spend even 10% of their time at depths exposed to tailings plumes (i.e., below 300m)

because their preferred habitat is in shallower depths of the Huon Gulf. For this reason, it is also unlikely that more than 10% of the diet of fish would comprise zooplankton and micronekton that have been potentially exposed to the DSTP subsurface plumes. If an area use factor of 10% (0.1) for the fish was used in these analyses, predicted fish tissue concentrations would be one tenth of those reported in Table 4-2. Applying an area use factor of 0.1, our results show that, apart from manganese, the presence of DSTP is predicted to not result in detectable change to metal concentrations already observed in fish from the Huon Gulf that are potentially consumed by people. Manganese is predicted to increase above the background range of <0.1 to 0.12mg/kg to 0.241mg/kg. This is due to the relatively high BAF at lower trophic levels measured in the samples. While the concentration of manganese is predicted to be double the observed background range, the predicted concentration is relatively low compared to amounts of manganese required in the human diet. The recommended adequate intake of manganese for adults 19 years and older is established as 2.3mg and 1.8mg per day for males and females, respectively (NAS, 2004). Adequate intakes range from 1.2mg to 1.6mg per day for children aged 1 to 18 years old. The tolerable upper intake level (i.e., the maximum usual daily intake level at which no risk of adverse health effects is expected) is 11mg per day for adults 19 and older and 2mg to 9mg per day for children aged 1 to 18. The USEPA determined an oral reference dose of 0.14mg/kg per day, which is an estimated dose that is not associated with adverse health effects in the general population (EPA, 2015). For a 70kg person, this value is 9.8mg per day of manganese. To exceed this value from fish consumption alone, one would need to eat in the order of 40kg of fish per day with a manganese concentration of 0.241mg/kg.

To further explore whether DSTP might have an effect on bioaccumulation of metals of interest, the TTFs reported in Brewer et al. (2012) for pelagic fish in the reference and mine-influenced locations in the Lihir DSTP site were examined, in comparison with the TTFs derived in the present analysis for the Huon Gulf applying a species area use factor of 0.1 (Table 4-3). As Brewer et al. (2012) report, there was little difference in bioaccumulation of metals in fish between samples from reference and mine-affected sites and, in general, fish tissue metals concentrations were similar between the reference and mine-influenced sites. Comparing the TTFs between studies, for most metals TTFs for fish at Lihir were similar to the TTFs derived for fish from the Huon Gulf (Table 4-3). The higher TTFs observed in some fish from the Huon Gulf may be due to limited number of fish species that could be used to derive site-specific TTFs, the amount of tissue data available for those species, and the (conservative) use of maximum tissue concentrations reported for each metal.

In an attempt to address this uncertainty, this assessment took the maximum tissue metal concentrations for fish that people consume (i.e. not including shark species) and derived an average tissue concentration for each metal (Table 3-7; Coffey, 2018c). The same approach for zooplankton and micronekton was used, involving averaging the maximum concentration reported for each species (Table 3-7; Coffey, 2018b) to derive an overall tissue concentration for both trophic levels and each metal (Table 4-4). This analysis supports the results presented in Tables 4-2 and 4-3. Tissue concentrations for copper, zinc, nickel, and manganese do not biomagnify in fish consumed by people. In contrast to the results reported by Brewer et al (2012), data collected thus far from the Huon Gulf suggest that arsenic and mercury may be bioaccumulated at higher concentrations in fish (Table 4-4). Furthermore, present conditions without DSTP in the Huon Gulf shows that some fish consumed by people in the Huon Gulf exceed food safety standards for arsenic and mercury (Table 4-4). The predicted arsenic (and other metal) concentrations in fish developed in this analysis and summarized in Table 4-2 do not account for sources of metals other than DSTP that influence observed metal concentrations in fish consumed by people. This omission does not appreciably affect the predictions for mercury, zinc, nickel, and manganese, due to the very low concentrations observed in marine existing waters. Operation of DSTP in the Huon Gulf is predicted to result in no change to the current arsenic and mercury concentrations in fish consumed by people.

As discussed in Section 3.1, the tissue metal concentrations estimated using trophic pathway modeling are likely to be conservative because this analysis assumes all species are continuously exposed to the subsurface plume. This is unlikely to be the case in the Huon Gulf, because many of these species either do not occur in the depths of the plumes, or migrate vertically and horizontally in the water column daily (see Section 2.4) and therefore, they are likely to be exposed to a subsurface plume for limited time periods and at more dilute concentrations than assumed.

Table 4-2: Predicted DSTP subsurface plume dissolved metal concentrations and predicted metal tissue concentrations (mg/kg, wet weight) in representative fish consumed in the Huon Gulf, assuming a DSTP discharge. The table includes a comparison with observed fish tissue concentrations and food safety standards (FSANZ 2011). NGA = no guideline available. Bold values exceed food safety standards.

Metal	Predicted DSTP Subsurface Plume Concentration (mg/L)	Fish Concentration from water ingestion only (mg/kg)	Fish Concentration from prey ingestion only (mg/kg)	Maximum Predicted Concentration Considering Area Use Factor of 0.1 (mg/kg)	Existing Concentrations in Fish Consumed by People in the Huon Gulf (mg/kg)	FSANZ Standard (mg/kg)
Arsenic	0.0016	**3.00**	**4.55**	0.455	<0.4 – **6.2**	2
Copper	0.011	**2.20**	**3.00**	0.3	0.11 – 0.50	2
Manganese	0.011	1.32	2.41	0.241	<0.1 – 0.12	NGA
Mercury	0.0000001	**0.53**	**0.53**	0.053	0.03 – **0.75**	0.5
Nickel	0.0014	0.07	0.087	0.0087	<0.06	NGA
Zinc	0.012	**5.53**	**7.18**	0.718	2.0 – 4.80	15

The predicted metals concentrations in fish do not include the very low background concentrations of metals in water. This omission does not appreciably affect the predictions for the six metals due to the very low background metals concentrations compared to the predicted metals concentrations in the DSTP subsurface plumes. Also, as the representative background tissue metals concentrations are calculated based on metals concentrations measured in the water (which were below detection limits), the maximum predicted concentrations in fish are due to DSTP only and not background concentrations in tissue.

Table 4-3: Comparison of trophic transfer factors (TTFs) based on Huon Gulf fish data and TTFs reported for reference and mining locations in the Lihir DSTP site (from Brewer et al 2012). Mercury was not reported in Brewer et al (2012). ND = no data.

Metal	Reference Sites (Brewer et al., 2012)	Mine sites (Brewer et al., 2012)	Fish Consumed by People from the Huon Gulf
Arsenic	0.64	0.67	0.45
Copper	0.11	0.07	0.30
Manganese	0.02	0.02	0.24
Nickel	0.07	0.07	0.01
Zinc	0.09	0.11	0.72
Mercury	ND	ND	0.05

Table 4-4: Tissue metal concentrations for zooplankton, micronekton, and fish species people consume from the Huon Gulf based on the average of maximum concentrations reported in Coffey (2018b; 2018c) for species in each trophic level.

	Copper (mg/kg)	Zinc (mg/kg)	Arsenic (mg/kg)	Mercury (mg/kg)	Nickel (mg/kg)	Manganese (mg/kg)
Zooplankton	1.76	6.63	2.17	0.01	0.16	4.40
Micronekton	4.30	11.16	0.96	0.04	0.41	2.45
Fish	0.20	3.18	2.86	0.41	0.03	0.06

5. Conclusions

The information reviewed for this evaluation indicates that fish consumed by people in the Huon Gulf may bioaccumulate metals of potential concern (i.e., arsenic, copper, manganese, mercury, nickel and zinc) via water and food ingestion from within the subsurface plumes from DSTP but not from benthic fauna or sediment. However, despite this assessment incorporating highly conservative assumptions in the predictions of metal bioaccumulation, the predictions indicate that concentrations of the metals of potential concern will generally not bioaccumulate or biomagnify to concentrations measurably above background ranges in fish presently consumed by people. The exception is manganese, which is predicted to increase to a concentration of 0.241mg/kg, which is above the observed background range (<0.1 to 0.12mg/kg). However, this concentration is an order of magnitude lower than daily concentrations required in the human diet and up to two orders of magnitude lower than concentrations where there are risks of adverse human health effects.

The analysis of trophic pathway modelling assumes that zooplankton, micronekton, fish that consume micronekton, and top trophic level fish are continuously exposed to subsurface plumes, and indicates that, with the exception of copper, DSTP is predicted to not result in metals concentrations exceeding Australian and New Zealand food safety standards. This is a highly conservative assumption, especially for the fish species consumed locally, which typically live at depths well above the depth of formation of subsurface plumes (i.e., above 300m), and the potential exposure to zooplankton and micronekton is only at the deepest part of the daytime downward migration. Therefore, when considering the appropriate species home ranges, no species consumed by humans are likely to spend as much as 10% of their time in the DSTP area (and probably much less time within the subsurface plumes), and considering this, none of the six metals examined is predicted to be increased significantly above background ranges nor exceed Australian and New Zealand food safety standards as a result of DSTP.

The predicted bioaccumulation results derived from these analyses are supported by other studies of metal bioaccumulation in fish in PNG and other tropical Asia-Pacific locations, and from metal bioaccumulation information from the published literature. Consistent with the findings of this assessment, these studies have found no bioaccumulation and biomagnification of metals concentrations beyond background concentrations in edible fish due to DSTP.

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Appendix A: Biota Information from the Huon Gulf

Table A-1: Summary of biota information from the Huon Gulf.

Type	Species	Trophic Level	Diet	Habitat	Min Depth (m)	Max Depth (m)	Reference
Zooplankton	Actinopyga mauritiana (Surf redfish)	2.1	substrate, algae/detritus	upper pelagic	0	20	Coffey 2018c
	Stichopus hermanni (Curryfish)	2.1	substrate, algae/detritus	upper pelagic	0	30	Coffey 2018c
	Holothuria atra (Lollyfish)	2.1	detritus and organic matter	upper pelagic	0	30	Coffey 2018c
	Bohadschia argus (Tigerfish/Leopardfish)	n/a	sand grains and detritus	upper pelagic	0	30	Coffey 2018c
	Actinopyga lecanora (Stonefish)	n/a	substrate, algae/detritus	upper pelagic	20	23	Coffey 2018c
	Thelenota ananas (Prickly redfish)	2.1	plants/detritus	upper pelagic	0	30	Coffey 2018c
	Holothuria coluber (Snakefish)	2.1	plants/detritus	upper pelagic	0	25	Coffey 2018c
	Holothuria fuscogilva (White teatfish)	2.1	plants/detritus	upper pelagic	0	40	Coffey 2018c
	Bohadschia similis (Brown-spotted sandfish)	2.1	plants/detritus	upper pelagic	3	16	Coffey 2018c
	Pearsonothuria graeffei (Flowerfish)	n/a	substrate, algae/detritus	upper pelagic	0	30	Coffey 2018c

Type	Species	Trophic Level	Diet	Habitat	Min Depth (m)	Max Depth (m)	Reference
	Thelenota anax (Amberfish)	n/a	substrate, algae/detritus	upper pelagic	10	30	Coffey 2018c
	Holothuria nobilis (Black testfish)	2.1	plants/detritus	upper pelagic	0	45	Coffey 2018c
	Stichopus chloronotus (Greenfish)	2.1	plants/detritus	upper pelagic	0	15	Coffey 2018c
	Actinopyga miliaris (Big blackfish)	2.1	plants/detritus	upper pelagic	0	20	Coffey 2018c
	Holothuria fuscopunctata (Elephant trunkfish)	2.1	plants/detritus	upper pelagic	0	25	Coffey 2018c
	Stichopus horrens (Dragonfish)	2.1	plants/detritus	upper pelagic	0	15	Coffey 2018c
	Holothuria edulis (Pinkfish)	2.1	plants/detritus	upper pelagic	0	45	Coffey 2018c
	Holothuria scabra (Sandfish)	2.4	plants/detritus	upper pelagic	0	10	Coffey 2018c
	Bohadschia vitiensis (Brown sandfish)	2.1	plants/detritus	upper pelagic	36	200	Coffey 2018c
	Lucifer	2.4	plants/detritus	upper pelagic	0	12	Coffey 2018d

Type	Species	Trophic Level	Diet	Habitat	Min Depth (m)	Max Depth (m)	Reference
	Microsetella	2.1	plants/detritus	lower and upper pelagic	0	767	Coffey 2018d
	Oikopleura	2.1	plants/detritus	upper pelagic	0	200	Coffey 2018d
	Acartia sinjiensis	2.4	algae/plants, detritus	upper pelagic	0	50	CSIRO 2018
	Echinometra mathaei	2.4	algae/plants, detritus	upper pelagic	0	139	CSIRO 2018
	Heliocidaris tuberculata (Tuberculate urchin)	n/a	plankton, benthos	upper pelagic	0	54	CSIRO 2018
	Aiptasia pulchella	n/a	n/a	upper pelagic	0	2	CSIRO 2018
Micronekton	Anguilla obscura (Pacific short-finned eel)	4.5	Crustaceans, finfish	upper and lower pelagic	0	3000	Coffey 2018c
	Myctophidae (Lanternfish)	3.1	zooplankton	lower and upper pelagic	25	900	Coffey 2018d
	Gonostomatidae (bristlemouths)	3.1	Euphausiids and copepods	lower and upper pelagic	0	4938	Coffey 2018d

Type	Species	Trophic Level	Diet	Habitat	Min Depth (m)	Max Depth (m)	Reference
	Trachichthyidae (slimehead)	4.3	benthos, crustaceans, mollusks, finfish	lower and upper pelagic	180	1809	Coffey 2018d
	Trachichthyid	4.3	benthos, crustaceans, mollusks, finfish	upper pelagic	0	250	Coffey 2018d
	Cavolinia	2.1	plants/detritus	lower and upper pelagic	0	1234	Coffey 2018d
	Clio	2.1	plants/detritus	lower and upper pelagic	0	600	Coffey 2018d
	Firoloida desmaresti	2.8	mainly animals	upper pelagic	0	100	Coffey 2018d
	Anguilliform	4.5	Crustaceans, finfish	upper pelagic	0	250	Coffey 2018d
	Bregmaceros sp.	3.2	plankton, crustaceans, phytoplankton	lower and upper pelagic	0	500	Coffey 2018d
	Chauliodus sloani	4.2	benthos, crustaceans, finfish	lower and upper pelagic	0	500	Coffey 2018d

Type	Species	Trophic Level	Diet	Habitat	Min Depth (m)	Max Depth (m)	Reference
	Conger eel	3.6	benthos, crustaceans, mollusks, insects, finfish	lower and upper pelagic	0	500	Coffey 2018d
	Decapoda A	n/a	n/a	lower and upper pelagic	0	500	Coffey 2018d
	Decapoda B	n/a	n/a	upper pelagic	0	250	Coffey 2018d
	Gonostomatid A	3.1	Euphausiids and copepods	upper pelagic	0	250	Coffey 2018d
	Gonostomatid B	3.1	Euphausiids and copepods	upper pelagic	0	250	Coffey 2018d
	Mysidacea	n/a	Phytoplankton, detritus, bacteria, protozoa	lower and upper pelagic	0	500	Coffey 2018d
	Pandalid	n/a	benthos, crustaceans, plankton, worms	lower pelagic	0	500	Coffey 2018d
	Pteropoda	n/a	n/a	lower and upper pelagic	0	500	Coffey 2018d

Type	Species	Trophic Level	Diet	Habitat	Min Depth (m)	Max Depth (m)	Reference
	Xenodermichthys nodulosus	3.4	n/a	lower and upper pelagic	0	500	Coffey 2018d
Fish	Centrophorus atromarginatus (dwarf gulper shark)	4.3	crustaceans, various deepwater fishes, shrimp, squids and jellyfish.	upper and lower pelagic	100	600	Coffey 2018b
	Centrophorus granulosus (gulper shark)	4.1	bony fish, cephalopods, and other invertebrates like crustaceans.	upper and lower pelagic	500	600	Coffey 2018b
	Centrophorus lusitanicus (Longfin gulper shark)	4.4	benthos, crustaceans, cephalopods, finfish	lower pelagic	300	500	Coffey 2018b
	Protonibea diacanthus (Blackspotted croaker)	3.5	benthos, crustaceans, finfish	upper pelagic	200	300	Coffey 2018b
	Lutjanus malabaricus (Saddletail snapper)	4.5	benthos, crustaceans, mollusks, worms, cephalopods, finfish	upper pelagic	30	200	Coffey 2018b
	Lutjanus argentimaculatus (Mangrove jack)	3.6	benthos, crustaceans, finfish	upper pelagic	0	200	Coffey 2018b
	Squalus crassispinus (Fatspine spurdog)	4.1	n/a	upper pelagic	100	300	Coffey 2018b
	Muraenesox bagio (Common pike eel)	4	benthos, crustaceans, finfish	upper pelagic	100	200	Coffey 2018b

Type	Species	Trophic Level	Diet	Habitat	Min Depth (m)	Max Depth (m)	Reference
	Alectis ciliaris (Pennantfish)	4	benthos, crustaceans, cephalopods, finfish	upper pelagic	30	40	Coffey 2018b
	Himantura granulata (Mangrove whipray)	4.1	benthos, crustaceans, worms, echinoderms, mollusks, finfish	upper pelagic	1	85	Coffey 2018c
	Muraenesox cinereus (Daggertooth pike conger)	4.4	benthos, crustaceans, finfish	upper and lower pelagic	0	800	Coffey 2018c
	Anodontostoma chacunda (Bony bream)	2.8	phytoplankton, detritus, mollusks, benthos, crustaceans, worms	upper pelagic	0	50	Coffey 2018c
	Sardinella melanura (Blacktip sardine)	2.8	n/a	upper pelagic	0	50	Coffey 2018c
	Sardinella albella (Perforated scale sardine)	2.6	Phytoplankton, plankton, crustaceans	upper pelagic	0	50	Coffey 2018c
	Setipinna tenuifilis (Hairfin anchovy)	3.6	benthos, crustaceans, mollusks, plankton, finfish	upper pelagic	0	50	Coffey 2018c
	Hemipimelodus sp. (Catfish)	3	benthos, insects, crustaceans	lower and upper pelagic	n/a	n/a	Coffey 2018c

Type	Species	Trophic Level	Diet	Habitat	Min Depth (m)	Max Depth (m)	Reference
	Netuma thalassina (Giant catfish)	3.5	benthos, crustaceans, worms, mollusks, finfish, cephalopods.	upper pelagic	0	195	Coffey 2018c
	Ambassis interruptus (Long-spined glass perchlet)	2.7	Plankton, crustaceans, mollusks, insects, detritus	upper pelagic	n/a	n/a	Coffey 2018c
	Fibramia amboinensis (Amboina cardinalfish)	3.7	Benthos and small fish	upper pelagic	2	5	Coffey 2018c
	Yarica hyalosoma (Humpbacked cardinalfish)	3.6	benthos, crustaceans, finfish	upper pelagic	0	2	Coffey 2018c
	Taeniamia buruensis (Buru cardinalfish)	3.5	Benthos and finfish	upper pelagic	0	3	Coffey 2018c
	Mesopristes cancellatus (Tapiroid grunter)	3	fish (early stages), benthos, crustaceans, phytoplankton, plankton	upper pelagic	2	9	Coffey 2018c
	Eleotris fusca (Dusky sleeper)	3.8	benthos, crustaceans, insects, finfish	upper pelagic	0	5	Coffey 2018c
	Eleotris macrolepis (Orange-bellied gudgeon)	3.7	Benthos and small fish	upper pelagic	0	5	Coffey 2018c
	Chelon macrolepis (Largescale mullet)	2.6	phytoplankton, mollusks, plankton, benthos, detritus	upper pelagic	1	10	Coffey 2018c

Type	Species	Trophic Level	Diet	Habitat	Min Depth (m)	Max Depth (m)	Reference
	Liza oligolepis (Broad-mouthed mullet)	2.5	Benthos and small fish	upper pelagic	1	2	Coffey 2018c
	Upeneus vittatus (Yellowstriped goatfish)	3.6	benthos, crustaceans, mollusks, echinoderms, worms, finfish	upper pelagic	0	100	Coffey 2018c
	Paraupeneus sp. (Goatfish species)	species dependent	Benthos and small fish	upper pelagic	n/a	n/a	Coffey 2018c
	Leiognathus equulus (Common ponyfish)	3	Plankton, invertebrates, benthos	upper pelagic	10	110	Coffey 2018c
	Gazza achlamys (Silvertooth ponyfish)	3.7	Benthos, crustaceans, worms, finfish	upper pelagic	0	20	Coffey 2018c
	Secutor runconius (Deep pugnose ponyfish)	2.7	zooplankton, phytoplankton, crustaceans	upper pelagic	3	60	Coffey 2018c
	Lactarius lactarius (False trevally)	4.2	fish (early stages), benthos, crustaceans, cephalopods, finfish	upper pelagic	15	100	Coffey 2018c
	Caranx sexfasciatus (Bigeye trevally)	4.5	benthos, crustaceans, finfish	upper pelagic	0	200	Coffey 2018c
	Lutjanus ehrenbergii (Blackspot snapper)	3.9	benthos, crustaceans, finfish	upper pelagic	5	20	Coffey 2018c

Type	Species	Trophic Level	Diet	Habitat	Min Depth (m)	Max Depth (m)	Reference
	Lutjanus johnii (Golden snapper)	4.2	benthos, crustaceans, mollusks, finfish	upper pelagic	0	80	Coffey 2018c
	Lutjanus lutjanus (Bigeye snapper)	4.1	benthos, crustaceans, finfish	upper pelagic	0	96	Coffey 2018c
	Lutjanus maxweberi (Pygmy snapper)	3.7	invertebrates and squid	upper pelagic	3	4	Coffey 2018c
	Lutjanus rivulatus (Blubberlip snapper)	4.1	benthos, crustaceans, finfish	upper pelagic	50	100	Coffey 2018c
	Lutjanus vaigiensis (Mangrove red snapper)	3.6	benthos, crustaceans, finfish	upper pelagic	1	120	Coffey 2018c
	Epinephelus daemelii (Saddled rockcod)	4	benthos, crustaceans, finfish	upper pelagic	0	50	Coffey 2018c
	Epinephelus tauvina (Greasy grouper)	4.1	benthos, crustaceans, finfish	upper pelagic	1	300	Coffey 2018c
	Gerres filamentosus (Whipfin silver-biddy)	3.4	crustaceans, polychaetes, worms, insect larvae	upper pelagic	1	22	Coffey 2018c
	Harpadon nehereus (Bombay-duck)	4.2	finfish	upper pelagic	50	400	Coffey 2018c
	Otolithes ruber (Tigertooth croaker)	3.6	benthos, crustaceans	upper pelagic	10	40	Coffey 2018c

Type	Species	Trophic Level	Diet	Habitat	Min Depth (m)	Max Depth (m)	Reference
	Johnius weberi (Weber's croaker)	3.4	n/a	upper pelagic	n/a	n/a	Coffey 2018c
	Lobotes surinamensis (Jumping Cod (tripletail))	4	benthos, crustaceans, finfish	upper pelagic	0	70	Coffey 2018c
	Platax orbicularis (Orbicular batfish)	3.3	benthos, algae/weeds, finfish	upper pelagic	5	30	Coffey 2018c
	Polydactylus microstomus (Small-mouthed threadfin)	3.9	benthos, crustaceans, finfish	upper pelagic	2	55	Coffey 2018c
	Pomadasys argyreus (Blue-checked javelinfish)	3.5	Crustaceans, fishes, polychaetes	upper pelagic	n/a	n/a	Coffey 2018c
	Scolopsis ghanam (Arabian monocle bream)	3.6	benthos, crustaceans, mollusks, echinoderms, finfish	upper pelagic	1	20	Coffey 2018c
	Scatophagus argus (Spotted scat)	3	benthos, crustaceans, phytoplankton, mollusks, worms, finfish	upper pelagic	0	5	Coffey 2018c
	Sicyopterus gymnauchen (Red-tailed goby)	2.8	phytoplankton, benthic algae, insects	upper pelagic	0	5	Coffey 2018c
	Toxotes jaculatrix (Banded archerfish)	2.6	Insects, plants	upper pelagic	0	2	Coffey 2018c

Type	Species	Trophic Level	Diet	Habitat	Min Depth (m)	Max Depth (m)	Reference
	Zenarchopterus dispar (Feathered river-garfish)	2.9	n/a	upper pelagic	0	3	Coffey 2018c
	Sphyrna zygaena (Hammerhead shark)	4.9	benthos, crustaceans, cephalopods, finfish	upper pelagic	0	200	Coffey 2018c
	Lutjanus sebae (Red emperor snapper)	4.1	benthos, crustaceans, cephalopods, finfish	upper pelagic	5	180	Coffey 2018c
	Elagatis bipinnulata (Rainbow runner)	4.3	benthos, crustaceans, mollusks, sponges/tunicates, plankton, finfish	upper pelagic	2	10	Coffey 2018c
	Naso hexacanthus (Sleek unicornfish)	3.1	detritus, plankton, crustaceans, jellyfish/hydroids	upper pelagic	6	150	Coffey 2018c
	Naso lopezi (Elongate unicornfish)	2.9	benthos, crustaceans, phytoplankton, mollusks	upper pelagic	20	50	Coffey 2018c
	Naso vlamingii (Bignose unifcornfish)	2.2	benthic algae/weeds, debris, invertebrates	upper pelagic	1	50	Coffey 2018c
	Canthidermis maculata (Rough triggerfish)	3.5	benthos, crustaceans, mollusks, tunicates, plankton, fish (early stages) hydroids	upper pelagic	1	110	Coffey 2018c

Type	Species	Trophic Level	Diet	Habitat	Min Depth (m)	Max Depth (m)	Reference
	Caesio cuning (Redbelly yellowtail fusilier)	3.4	zooplankton, invertebrates	upper pelagic	1	60	Coffey 2018c
	Carangoides bajad (Orange-spotted trevally)	4	benthos, crustaceans, finfish	upper pelagic	2	70	Coffey 2018c
	Carangoides plagiotaenia (Barcheek trevally)	4	benthos, crustaceans, finfish	upper pelagic	2	200	Coffey 2018c
	Caranx melampygus (Bluefin trevally)	4.5	benthos, crustaceans, mollusks, finfish	upper pelagic	0	190	Coffey 2018c
	Caranx papuensis (Brassy trevally)	4	benthos, crustaceans, finfish	upper pelagic	1	50	Coffey 2018c
	Platax pinnatus (Dusky batfish)	3.3	benthos, invertebrates, jellyfish/hydroids, plankton	upper pelagic	15	30	Coffey 2018c
	Platax teira (Longfin batfish)	4	benthos, crustaceans, finfish	upper pelagic	3	25	Coffey 2018c
	Diagramma pictum (Painted sweetlips)	3.7	benthos, crustaceans, mollusks, finfish, worms	upper pelagic	1	170	Coffey 2018c
	Plectorhinchus lineatus (Yellow-banded sweetlips)	3.9	benthos, crustaceans, finfish	upper pelagic	1	35	Coffey 2018c
	Myripristis adusta (Shadowfin soldierfish)	3.4	plankton, invertebrates	upper pelagic	1	25	Coffey 2018c

Type	Species	Trophic Level	Diet	Habitat	Min Depth (m)	Max Depth (m)	Reference
	Myripristis kuntee (Shoulderbar soldierfish)	3.4	mysids, plankton, crustaceans, copepods, polychaetes, stomapods, bony fish	upper pelagic	0	65	Coffey 2018c
	Myripristis violacea (Lattice soldierfish)	3.5	benthos, crustaceans, plankton, worms, finfish	upper pelagic	3	30	Coffey 2018c
	Myripristis vittata (Whitetip soldierfish)	3.8	Benthos, crustaceans, plankton, finfish	upper pelagic	3	80	Coffey 2018c
	Neoniphon sammara (Sammara squirrelfish)	3.6	benthos, crustaceans, finfish	upper pelagic	0	46	Coffey 2018c
	Sargocentron caudimaculatum (Silverspot squirrelfish)	3.9	benthos, crustaceans, finfish	upper pelagic	2	40	Coffey 2018c
	Kyphosus cinerascens (Blue sea chub)	2.9	benthic algae/weeds, benthos, crustaceans, finfish	upper pelagic	1	45	Coffey 2018c
	Kyphosus vaigiensis (Brassy chub)	2	benthic algae/weeds, debris, invertebrates	upper pelagic	0	40	Coffey 2018c
	Lethrinus erythropterus (Longfin emperor)	3.7	Benthos, crustaceans, echinoderms, finfish	upper pelagic	2	25	Coffey 2018c
	Monotaxis grandoculis (Humpnose big-eye bream)	3.4	benthos, crustaceans, echinoderms, mollusks, finfish	upper pelagic	1	100	Coffey 2018c

Type	Species	Trophic Level	Diet	Habitat	Min Depth (m)	Max Depth (m)	Reference
	Lutjanus biguttatus (Two-spot banded snapper)	4	benthos, crustaceans, finfish	upper pelagic	3	36	Coffey 2018c
	Lutjanus boutton (Molucccan snapper)	3.8	benthos, crustaceans, cephalopods, finfish	upper pelagic	3	50	Coffey 2018c
	Lutjanus carponotatus (Stripey snapper)	3.9	benthos, crustaceans, finfish	upper pelagic	1	80	Coffey 2018c
	Lutjanus fulvus (Blacktail snapper)	3.6	Benthos, crustaceans, worms, finfish	upper pelagic	1	75	Coffey 2018c
	Lutjanus gibbus (Humpback red snapper)	4.1	benthos, crustaceans, mollusks, echinoderms, finfish	upper pelagic	1	150	Coffey 2018c
	Lutjanus kasmira (Bluestriped snapper)	3.9	Benthos, crustaceans, mollusks, finfish	upper pelagic	3	265	Coffey 2018c
	Lutjanus monostigma (One-spot snapper)	4.3	benthos, crustaceans, finfish	upper pelagic	1	60	Coffey 2018c
	Lutjanus rivulatus (Blubberlip snapper)	4.1	benthos, crustaceans, cephalopods, finfish	upper pelagic	50	100	Coffey 2018c
	Lutjanus russellii (Moses' snapper)	4.1	benthos, crustaceans, mollusks, cephalopods, finfish	upper pelagic	3	80	Coffey 2018c

Type	Species	Trophic Level	Diet	Habitat	Min Depth (m)	Max Depth (m)	Reference
	Lutjanus semicinctus (Blackbanded snapper)	4.1	benthos, crustaceans, finfish	upper pelagic	10	36	Coffey 2018c
	Lutjanus vitta (Brownstripe snapper)	4	Benthos, crustaceans, cephalopods, finfish	upper pelagic	10	72	Coffey 2018c
	Macolor niger (Black-and-white snapper)	4	benthos, crustaceans, finfish	upper pelagic	2	90	Coffey 2018c
	Macolor macularis (Midnight snapper)	4	benthos, crustaceans, plankton, finfish	upper pelagic	3	90	Coffey 2018c
	Mulloidichthys vanicolensis (Yellowfin goatfish)	3.6	benthos, crustaceans, worms, finfish	upper pelagic	1	113	Coffey 2018c
	Parupeneus barberinus (Dash-and-dot goatfish)	3.4	benthos, crustaceans, plankton, mollusks, echinoderms, worms	upper pelagic	1	100	Coffey 2018c
	Parupeneus cyclostomus (Gold-saddle goatfish)	4.2	benthos, crustaceans, finfish	upper pelagic	2	125	Coffey 2018c
	Parupeneus multifasciatus (Manybar goatfish)	3.5	benthos, crustaceans, finfish	upper pelagic	3	161	Coffey 2018c
	Parupeneus trifasciatus (Doublebar goatfish)	3.5	benthos, crustaceans, finfish	upper pelagic	1	80	Coffey 2018c
	Scarus flavipectoralis (Yellowfin parrotfish)	2	Benthic algae/weeds	upper pelagic	2	40	Coffey 2018c

Type	Species	Trophic Level	Diet	Habitat	Min Depth (m)	Max Depth (m)	Reference
	Gymnosarda unicolor (Dogtooth tuna)	4.5	Cephalopods, finfish	upper pelagic	10	250	Coffey 2018c
	Scomberomorus commerson (Narrow-barred Spanish mackerel)	4.5	benthos, crustaceans, finfish	upper pelagic	10	70	Coffey 2018c
	Anyperodon leucogrammicus (Slender grouper)	3.9	benthos, crustaceans, finfish	upper pelagic	5	80	Coffey 2018c
	Cephalopholis boenak (Brown-barred rockcod)	4.1	benthos, crustaceans, finfish	upper pelagic	1	64	Coffey 2018c
	Cephalopholis cyanostigma (Blue-spotted rockcod)	4.2	benthos, crustaceans, finfish	upper pelagic	1	50	Coffey 2018c
	Cephalopholis microprion (Dot-head rockcod)	4	benthos, crustaceans, finfish	upper pelagic	0	52	Coffey 2018c
	Cephalopholis sexmaculata (Sixband rockcod)	4	benthos, crustaceans, finfish	upper pelagic	6	150	Coffey 2018c
	Cephalopholis urodeta (Flagtail rockcod)	4	benthos, crustaceans, finfish	upper pelagic	1	60	Coffey 2018c
	Plectropomus areolatus (Spotted coral trout)	4.5	finfish	upper pelagic	1	20	Coffey 2018c
	Plectropomus leopardus (Leopard coral trout)	4.4	benthos, crustaceans, cephalopods, finfish	upper pelagic	3	100	Coffey 2018c

Type	Species	Trophic Level	Diet	Habitat	Min Depth (m)	Max Depth (m)	Reference
	Plectropomus oligacanthus (Vermicular cod)	4	benthos, crustaceans, finfish	upper pelagic	5	147	Coffey 2018c
	Siganus javus (Java rabbitfish)	2.4	plankton, phytoplankton, jellyfish/hydroids, detritus	upper pelagic	0	20	Coffey 2018c
	Siganus lineatus (Gold-lined rabbitfish)	2	benthic algae/weeds	upper pelagic	0	25	Coffey 2018c
	Siganus puellus (Masked rabbitfish)	3	sponges/tunicates	upper pelagic	1	30	Coffey 2018c
	Pristipomoides typus (sharptooth jobfish)	4.2	benthos, crustaceans, cephalopods, finfish	upper pelagic	30	200	Coffey 2018c
	Scomber australasicus (Blue mackerel)	4.2	plankton, crustaceans, fish (early stages), finfish, cephalopods worms	upper pelagic	87	200	Coffey 2018c
	Coryphaena hippurus (dolphinfish)	4.4	Plankton, crustaceans, finfish, cephalopods	upper pelagic	0	85	Coffey 2018c
	Scomberomorus commerson (Spanish mackerel)	4.5	benthic, crustaceans, plankton, cephalopods, finfish	upper pelagic	10	70	Coffey 2018c
	Acanthocybium solandri (Wahoo)	4.3	cephalopods, mollusks, finfish	upper pelagic	0	20	Coffey 2018c

Type	Species	Trophic Level	Diet	Habitat	Min Depth (m)	Max Depth (m)	Reference
	Istiompax indica (Black marlin)	4.5	cephalopods, mollusks, finfish	lower and upper pelagic	0	915	Coffey 2018c
	Makaira mazara (Blue marlin)	4.5	benthos, crustaceans, mollusks, cephalopods, finfish	upper pelagic	0	200	Coffey 2018c
	Istiophorus platypterus (Sailfish)	4.5	benthos, crustaceans, cephalopods, finfish	upper pelagic	0	200	Coffey 2018c
	Thunnus obesus (Bigeye tuna)	4.5	benthos, crustaceans, cephalopods, finfish	upper pelagic	0	250	Coffey 2018c
	Thunnus alalunga (Albacore tuna)	4.3	benthos, crustaceans, cephalopods, finfish	lower and upper pelagic	0	600	Coffey 2018c
	Thunnus maccoyii (Southern bluefin tuna)	3.9	benthos, crustaceans, mollusks, plankton, cephalopods, finfish	lower and upper pelagic	50	2743	Coffey 2018c
	Thunnus orientalis (Pacific bluefin tuna)	4.5	benthos, crustaceans, plankton, mollusks, finfish	upper pelagic	1	200	Coffey 2018c
	Thunnus albacares (Yellowfin tuna)	4.4	benthos, crustaceans, plankton, cephalopods, mollusks, finfish	upper pelagic	1	250	Coffey 2018c

Type	Species	Trophic Level	Diet	Habitat	Min Depth (m)	Max Depth (m)	Reference
	Katsuwonus pelamis (Skipjack tuna)	4.4	benthos, crustaceans, plankton, cephalopods, mollusks, finfish	upper pelagic	0	260	Coffey 2018c
	Seriola lalandi (Yellowtail kingfish)	4.2	benthos, crustaceans, cephalopods, finfish	upper and lower pelagic	3	825	CSIRO 2018

Appendix B: Metal Data for Tailings, Tailings Liquor, and Laboratory Toxicity Tests; Zooplankton, Micronekton, and Fish Tissue Metal

Table B-1: Summary of Metal Data for Tailings, Tailings Liquor, and Laboratory Toxicity Tests; Zooplankton, Micronekton, and Fish Tissue Metal Data for Organisms Collected in Huon Gulf.

Tailings sample type	Species of tissue sample	Sample Date	Depth (m)	Copper (µg/L)		Zinc (µg/L)		Arsenic (µg/L)		Mercury (µg/L)		Nickel (µg/L)		Manganese (µg/L)		Ref.
				Total	Diss.	Total	Diss.	Total	Diss.	Total	Methyl-	Total	Diss.	Total	Diss.	
fish tissue	Dwarf gulper shark	Nov-16	100-600	0.12 - 0.18		2.10 - 3.70		6.9 - 36		0.02 - 2.20		<0.06 - 0.07		<0.1 - 0.14		a
fish tissue	Longfin gulper shark	Nov-16	300-400	0.16 - 0.25		2.30 - 2.70		12 - 34		0.99 - 1.30		<0.06 - <0.06		0.10 - 0.11		a
fish tissue	Blackspotted croaker	Nov-16	200-300	0.13		2.2		0.96		0.26		<0.06		<0.1		a
fish tissue	Gulper shark	Nov-16	500-600	0.11		3.30		33		1.50		<0.06		<0.1		a
fish tissue	Mangrove jack	Nov-16	0-70	0.13		3.10		<0.4		0.53		<0.06		<0.1		a
fish tissue	Saddletail snapper	Nov-16	0-200	0.11		2.20		2.8		0.05		<0.06		<0.1		a
fish tissue	Mangrove jack	Nov-16	100-200	0.11 - 0.12		2.60 - 2.80		0.41 - 2.6		0.03 - 0.05		<0.06 - <0.06		<0.1 - <0.1		
fish tissue	Saddletail snapper	Nov-16	30-200	0.08 - 0.10		2.20 - 3.40		2.3 - 6.2		0.19 - 0.36		<0.06 - <0.06		<0.1 - 0.10		a
fish tissue	Sharptooth jobfish	Nov-16	30-200	0.11 - 0.14		2.40 - 2.50		0.83 - 1.80		0.05 - 0.35		<0.06 - <0.06		<0.1 - <0.1		a
fish tissue	Bigeye trevally	Nov-16	0-200	0.29 - 0.50		3.40 - 4.80		<0.4 - 0.60		0.34 - 0.71		<0.06 - <0.06		<0.1 - 0.11		a
fish tissue	Pennantfish	Nov-16	30-40	0.19		3.10		5.0		0.25		<0.06		<0.1		a

Tailings sample type	Species of tissue sample	Sample Date	Depth (m)	Copper (µg/L)		Zinc (µg/L)		Arsenic (µg/L)		Mercury (µg/L)		Nickel (µg/L)		Manganese (µg/L)		Ref.
				Total	Diss.	Total	Diss.	Total	Diss.	Total	Methyl-	Total	Diss.	Total	Diss.	
fish tissue	Dwarf gulper shark	May-17	100-400	0.07 - 0.10		1.90 - 2.40		8.9 - 30		0.14 - 1.10		<0.06 - <0.06		<0.1 - 0.18		a
fish tissue	Longfin gulper shark	May-17	300-500	0.09 - 0.11		2.10 - 2.70		16 - 38		0.35 - 0.97		<0.06 - <0.06		<0.1 - <0.1		a
fish tissue	Fatspine spurdog	May-17	100-300	0.11 - 0.16		2.0 - 2.50		5.5 - 21		0.08 - 0.75		<0.06 - <0.06		<0.1 - <0.1		a
fish tissue	Saddletail snapper	May-17	100-200	0.09		2.80		3.7		0.06		<0.06		<0.1		a
fish tissue	Common pike eel	May-17	100-200	0.10		2.60		32		0.24		<0.06		0.12		a
zooplankton	not given	Mar-17	0-100	0.6 - 2.3		2.6 - 7.1		0.82 - 3.0		<0.01		0.1-0.21		0.97 - 8.02		b
zooplankton	not given	Mar-17	0-250	0.42 - 2.4		1.7 - 8.6		0.47 - 1.9		<0.01		0.08 - 0.17		0.56 - 1.95		b
zooplankton	not given	Mar-17	0-500	0.16 - 0.58		1.2 - 4.2		<0.4 - 1.6		<0.01		<0.06 - 0.17		0.41 - 3.23		b
Micronekton	Decapoda B	May-17	0-250	5.8		11		0.43		0.02		<0.1		1.1		b
Micronekton	Gonostomatid A	May-17	0-250	2.2 - 4.8		10-18		0.29 - 0.64		0.01 - 0.01		0.24 - 1.3		1.7 - 2.1		b
Micronekton	Gonostomatid B	May-17	0-250	3.4		13		0.9		0.01		0.36		3.2		b

Tailings sample type	Species of tissue sample	Sample Date	Depth (m)	Copper (µg/L)		Zinc (µg/L)		Arsenic (µg/L)		Mercury (µg/L)		Nickel (µg/L)		Manganese (µg/L)		Ref.
				Total	Diss.	Total	Diss.	Total	Diss.	Total	Methyl-	Total	Diss.	Total	Diss.	
Micronekton	Pandalid	May-17	0-250	17.0		8.1		1.0		0.03		<0.1		1.5		b
Micronekton	Anguilliform	May-17	0-250	1.4 - 3.0		6.2 - 8.5		0.26 - 0.45		0.02 - 0.02		0.14 - 0.14		1.3 - 2.7		b
Micronekton	Trachichthyid	May-17	0-250	2.5		8.8		0.52		0.03		0.25		2.7		b
Micronekton	Pandalid	May-17	0-500	17.0		9.1		0.65		0.03		0.16		2.0		b
Micronekton	Bregmaceros sp.	May-17	0-500	1.4 - 4.0		11-16		0.33 - 0.36		0.02 - 0.03		0.25 - 1.1		3.0 - 3.2		b
Micronekton	Chauliodus sloani	May-17	0-500	0.34 - 0.46		2.9 - 4.6		0.5 - 0.59		0.02 - 0.02		<0.1 - <0.1		0.65 - 0.94		b
Micronekton	Conger eel	May-17	0-500	1.8		6.6		0.44		0.13		<0.1		0.34		b
Micronekton	Decapoda A	May-17	0-500	1.7		7.3		0.19		0.02		0.54		0.83		b
Micronekton	Mysidacea	May-17	0-500	4.8		12		0.24		0.01		0.14		1.6		b
Micronekton	Pteropoda	May-17	0-500	3.8		7.2		0.37		0.1		0.97		8.8		b
Micronekton	Xenodermichthys nodulosus	May-17	0-500	1.4 - 2.9		19 - 23		5.0 - 6.4		0.03-0.04		0.13 - 0.18		1.6 - 2.3		b
bivalve tissue	Tellina deltoidalis	early 2016		450 ± 360		320 ± 76		15 ± 8.5				8.1 ± 5.8		56 ± 43		c
bivalve tissue	Tellina deltoidalis	late 2016		350 ± 150		550 ± 400		16 ± 7.9				25 ± 23		210 ± 220		c

Tailings sample type	Species of tissue sample	Sample Date	Depth (m)	Copper (µg/L)		Zinc (µg/L)		Arsenic (µg/L)		Mercury (µg/L)		Nickel (µg/L)		Manganese (µg/L)		Ref.
				Total	Diss.	Total	Diss.	Total	Diss.	Total	Methyl-	Total	Diss.	Total	Diss.	
control/ background sediment		2/20/2017	355	74	no data	79	no data	13	no data	no data		71	no data	1600	no data	d
control/ background sediment		2/20/2017	589	60	4.1	69	<5.0	6	<1.00	<0.1		37	<1.0	799	51	d
control/ background sediment		2/20/2017	654	95	17.0	94	<5.0	11	<1.00	<0.1		60	<1.0	1180	200	d
control/ background sediment		2/20/2017	721	48	4.4	59	<5.0	6	<1.00	<0.1		34	<1.0	664	58	d
control/ background sediment		2/21/2017	1098	54	3.5	62	<5.0	<5	<1.00	<0.1		33	<1.0	766	44	d
control/ background sediment		2/21/2017	1143	85	10.2	91	<5.0	10	<1.00	<0.1		56	<1.0	1040	121	d
control/ background sediment		2/21/2017	1022	91	16.3	100	<5.0	14	<1.00	<0.1		63	<1.0	1250	194	d

Tailings sample type	Species of tissue sample	Sample Date	Depth (m)	Copper (µg/L)		Zinc (µg/L)		Arsenic (µg/L)		Mercury (µg/L)		Nickel (µg/L)		Manganese (µg/L)		Ref.
				Total	Diss.	Total	Diss.	Total	Diss.	Total	Methyl-	Total	Diss.	Total	Diss.	
control/ background sediment		2/21/2017	915	51	3.0	61	<5.0	5	<1.00	<0.1		32	<1.0	732	39	d
control/ background sediment		2/23/2017	2121	66	16.1	81	<5.0	10	<1.00	<0.1		50	<1.0	1140	370	d
control/ background sediment		2/23/2017	2001	66	12.3	69	<5.0	7	<1.00	<0.1		46	<1.0	823	112	d
control/ background sediment		2/23/2017	1489	63	4.5	64	<5.0	5	<1.00	<0.1		40	<1.0	808	47	d
control/ background sediment		2/23/2017	1341	55	3.4	59	<5.0	<5	<1.00	<0.1		35	<1.0	727	40	d
control/ background sediment		2/23/2017	1656	92	13.6	79	6.8	9	<1.00	<0.1		103	6.0	5520	1520	d
solid tailings		early 2016		915 - 1570		472 - 840		13 - 27		0.02 - 0.03		234 - 299		300 - 366		c
solid tailings		late 2016		929 - 1050		493 - 552		14 - 16		<0.02 - 0.02		274 - 305		296 - 308		c

Tailings sample type	Species of tissue sample	Sample Date	Depth (m)	Copper (µg/L)		Zinc (µg/L)		Arsenic (µg/L)		Mercury (µg/L)		Nickel (µg/L)		Manganese (µg/L)		Ref.
				Total	Diss.	Total	Diss.	Total	Diss.	Total	Methyl-	Total	Diss.	Total	Diss.	
tailings liquor		early 2016			69		145		0.4	no data			34		2020	c
tailings liquor		early 2016			69		145		0.4	no data			34		2020	c
tailings liquor		early 2016			69		145		0.4	no data			34		2020	c
tailings liquor		early 2016			69		145		0.4	no data			34		2020	c
tailings liquor		early 2016			69		145		0.4	no data			34		2020	c
tailings liquor		early 2016			69		145		0.4	no data			34		2020	c
tailings liquor		early 2016			69		145		0.4	no data			34		2020	c
tailings liquor		early 2016			69		145		0.4	no data			34		2020	c
tailings liquor		early 2016			69		145		0.4	no data			34		2020	c
tailings liquor		early 2016			69		145		0.4	no data			34		2020	c
tailings liquor		early 2016			69		145		0.4	no data			34		2020	c

Tailings sample type	Species of tissue sample	Sample Date	Depth (m)	Copper (µg/L)		Zinc (µg/L)		Arsenic (µg/L)		Mercury (µg/L)		Nickel (µg/L)		Manganese (µg/L)		Ref.
				Total	Diss.	Total	Diss.	Total	Diss.	Total	Methyl-	Total	Diss.	Total	Diss.	
tailings liquor		early 2016			69		145		0.4	no data			34		2020	c
tailings liquor		early 2016			69		145		0.4	no data			34		2020	c
tailings liquor		early 2016			69		145		0.4	no data			34		2020	c
tailings liquor		early 2016			69		145		0.4	no data			34		2020	c
tailings liquor		early 2016			69		145		0.4	no data			34		2020	c
tailings liquor		early 2016			69		145		0.4	no data			34		2020	c
tailings liquor		early 2016			69		145		0.4	no data			34		2020	c
tailings liquor		early 2016			69		145		0.4	no data			34		2020	c
tailings liquor		early 2016			69		145		0.4	no data			34		2020	c
tailings liquor		early 2016			69		145		0.4	no data			34		2020	c
tailings liquor		early 2016			69		145		0.4	no data			34		2020	c

Tailings sample type	Species of tissue sample	Sample Date	Depth (m)	Copper (µg/L)		Zinc (µg/L)		Arsenic (µg/L)		Mercury (µg/L)		Nickel (µg/L)		Manganese (µg/L)		Ref.
				Total	Diss.	Total	Diss.	Total	Diss.	Total	Methyl-	Total	Diss.	Total	Diss.	
tailings liquor		early 2016			69		145		0.4	no data			34		2020	c
tailings liquor		early 2016			69		145		0.4	no data			34		2020	c
tailings liquor		late 2016			19		287		0.9	no data			62		3160	c
tailings liquor		late 2016			19		287		0.9	no data			62		3160	c
tailings liquor		late 2016			19		287		0.9	no data			62		3160	c
tailings liquor		late 2016			19		287		0.9	no data			62		3160	c
tailings liquor		late 2016			19		287		0.9	no data			62		3160	c
tailings liquor		late 2016			19		287		0.9	no data			62		3160	c
tailings liquor		late 2016			19		287		0.9	no data			62		3160	c
tailings liquor		late 2016			19		287		0.9	no data			62		3160	c
tailings liquor		late 2016			19		287		0.9	no data			62		3160	c

Tailings sample type	Species of tissue sample	Sample Date	Depth (m)	Copper (µg/L)		Zinc (µg/L)		Arsenic (µg/L)		Mercury (µg/L)		Nickel (µg/L)		Manganese (µg/L)		Ref.
				Total	Diss.	Total	Diss.	Total	Diss.	Total	Methyl-	Total	Diss.	Total	Diss.	
tailings liquor		late 2016			19		287		0.9	no data			62		3160	c
tailings liquor		late 2016			19		287		0.9	no data			62		3160	c
tailings liquor		late 2016			19		287		0.9	no data			62		3160	c
tailings liquor		late 2016			19		287		0.9	no data			62		3160	c
tailings liquor		late 2016			19		287		0.9	no data			62		3160	c
tailings liquor		late 2016			19		287		0.9	no data			62		3160	c
tailings liquor		late 2016			19		287		0.9	no data			62		3160	c
tailings liquor		late 2016			19		287		0.9	no data			62		3160	c
tailings liquor		late 2016			19		287		0.9	no data			62		3160	c
tailings liquor		late 2016			19		287		0.9	no data			62		3160	c
tailings liquor		late 2016			19		287		0.9	no data			62		3160	c

Tailings sample type	Species of tissue sample	Sample Date	Depth (m)	Copper (µg/L)		Zinc (µg/L)		Arsenic (µg/L)		Mercury (µg/L)		Nickel (µg/L)		Manganese (µg/L)		Ref.
				Total	Diss.	Total	Diss.	Total	Diss.	Total	Methyl-	Total	Diss.	Total	Diss.	
tailings liquor		late 2016			19		287		0.9	no data			62		3160	c
tailings liquor		late 2016			19		287		0.9	no data			62		3160	c
tailings liquor		late 2016			19		287		0.9	no data			62		3160	c
tailings liquor		late 2016			19		287		0.9	no data			62		3160	c

NOTE: Control samples do not have tailings of any kind. Acid extractable metal = dissolved metal for this table All concentrations should be as either mg/kg or mg/L. min - max concentrations are presented unless n=1. tissue samples were analysed by AAA (NSW) for total trace concentrations (mg/kg-1) (wet weight concentrations)

a - Deep-slope and pelagic fish characterisation study. Coffey, 2018a

b - Zooplankton and micronekton characterisation study. Coffey, 2018b

c - Chemistry and ecotoxicology characterisation of pilot-scale tailing for the Wafi Golpu, Papua New Guinea. CSIRO, 2018

d - WAFI-GOLPU PROJECT Physical, Chemical and Biological Sedimentology of the Huon Gulf

Appendix C: Bioaccumulation Calculations in Different Trophic Levels Based on Trophic Pathway Modeling

Table C-1: Summary of modeled water and tissue concentrations of concern for three different trophic levels: zooplankton (trophic level 1), micronekton (trophic level 2), and fish that people consume

Scenario	Chemical	Dissolved Water Concentration (ug/L)	Zooplankton Concentration (mg/kg)	Micronekton Concentration (mg/kg)	Fish Concentration (mg/kg)
Tailings Liquor 1	Arsenic	1.5	3.5	2.7	4.5
	Copper	11	26.4	251.5	3.0
	Manganese	11	176.4	291.8	2.4
	Mercury	0.0001	0.01	0.26	0.5
	Nickel	1.1	0.46	4.04	0.08
	Zinc	8.7	14.9	56.5	7.2
Tailings Liquor 2	Arsenic	1.6	3.68	2.8	4.8
	Copper	11	26.4	251.5	3.0
	Manganese	8	128.3	212.2	1.7
	Mercury	0.0001	0.01	0.26	0.53
	Nickel	1.4	0.58	5.2	0.11
	Zinc	12	20.6	77.9	9.9

Table C-2: Modeled zooplankton tissue concentrations

Scenario	Chemical	Zooplankton BCF (mg Chemical/kg wet tissue)/(ug chemical/L)	Water Concentration (ug/L)	Zooplankton Concentration (mg Chemical/kg wet tissue)
Tailings Liquor 1	Arsenic	2.3	1.5	3.5
	Copper	2.4	11.0	26.4
	Manganese	16.0	11.0	176.4
	Mercury	100.0	0.00	0.01
	Nickel	0.4	1.1	0.5
	Zinc	1.7	8.7	15.0
Tailings Liquor 2	Arsenic	2.3	1.6	3.7
	Copper	2.4	11.0	26.4
	Manganese	16.0	8.0	128.3
	Mercury	100.0	0.00	0.01
	Nickel	0.4	1.4	0.6
	Zinc	1.7	12.0	20.6

Table C-3: Modeled micronekton tissue concentrations

Scenario	Chemical	Micronekton BCF (mg Chemical/kg wet tissue)/(ug chemical/L)	Water Concentration (ug/L)	Micronekton Concentration from Water	Micronekton TTF (mg Chemical/kg wet tissue)/(ug chemical/L)	Zooplankton Concentration	Micronekton Concentration from Diet
Tailings Liquor 1	Arsenic	0.77	1.50	1.16	0.44	3.45	1.53
	Copper	17.00	11.00	187.00	2.44	26.40	64.50
	Manganese	17.60	11.00	193.60	0.56	176.44	98.25
	Mercury	1300.00	0.00	0.13	13.00	0.01	0.13
	Nickel	2.60	1.10	2.86	2.56	0.46	1.18
	Zinc	3.60	8.70	31.32	1.68	14.96	25.19
Tailings Liquor 2	Arsenic	0.77	1.60	1.23	0.44	3.68	1.63
	Copper	17.00	11.00	187.00	2.44	26.40	64.50
	Manganese	17.60	8.00	140.80	0.56	128.32	71.45
	Mercury	1300.00	0.00	0.13	13.00	0.01	0.13
	Nickel	2.60	1.40	3.64	2.56	0.59	1.51
	Zinc	3.60	12.00	43.20	1.68	20.64	34.74

Table C-4: Modeled Fish tissue concentrations

Scenario	Chemical	Fish BCF (mg Chemical/kg wet tissue)/(ug chemical/L)	Water Concentration (ug/L)	Fish Concentration from Water	Micronekton Concentration (mg Chemical/kg wet tissue)	Fish TTF (mg Chemical/kg wet tissue)/(mg chemical/kg)	Fish Concentration from Ingestion of Micronekton
Tailings Liquor 1	Arsenic	2.00	1.50	3.00	1.53	2.98	4.55
	Copper	0.20	11.00	2.20	64.50	0.05	3.00
	Manganese	0.12	11.00	1.32	98.25	0.02	2.41
	Mercury	5300.00	0.00	0.53	0.13	4.08	0.53
	Nickel	0.06	1.10	0.07	1.18	0.07	0.09
	Zinc	0.64	8.70	5.53	25.19	0.28	7.18
Tailings Liquor 2	Arsenic	2.00	1.60	3.20	1.63	2.98	4.85
	Copper	0.13	11.00	1.43	64.50	0.05	3.00
	Manganese	0.20	8.00	1.60	71.45	0.02	1.75
	Mercury	5300.00	0.00	0.53	0.13	4.08	0.53
	Nickel	0.12	1.40	0.17	1.51	0.07	0.11
	Zinc	0.62	12.00	7.44	34.74	0.28	9.90

Appendix D: USEPA Methodology for Predicting Metal Concentrations in Fish

United States Environmental Protection Agency | Solid Waste and Emergency Response (5305W) | EPA530-D-99-001A August 1999 www.epa.gov/osw

Screening Level Ecological Risk Assessment Protocol for Hazardous Waste Combustion Facilities

Volume One

Peer Review Draft

5.3.2 COPC Concentrations in Food Items of Measurement Receptors

Determination of COPC concentrations in food items is required for calculating the daily dose of COPC ingested for each class-specific guild measurement receptor being evaluated. Since the risk assessment considers potential future exposure that may occur as a result of facility emissions over time, these concentrations are generally expected to be estimated mathmatically. The following subsections provide guidance for estimating COPC concentrations in the following groups of food items:

- Invertebrates, phytoplankton, and rooted aquatic plants;
- Terrestrial plants;
- Fish; and
- Mammals, birds, reptiles, and amphibians.

5.3.2.1 COPC Concentration in Invertebrates, Phytoplankton, and Rooted Aquatic Plants

COPC concentrations in invertebrate, phytoplankton, and rooted aquatic plants can be calculated by rearranging the mathmatical expression for a bioconcentration factor (*BCF*). Equation 5-2 is the mathmatical definition of a *BCF*, which is the ratio, at steady-state, of the concentration of a compound in a food item to its concentration in a media. Equation 5-3 is the same equation expressed in terms of a COPC concentration in a food item.

$$BCF = \frac{C_i}{C_M} \qquad \text{Equation 5-2}$$

$$C_i = C_M \cdot BCF \qquad \text{Equation 5-3}$$

where

BCF	=	Bioconcentration factor (unitless [soil, sediment], or L/kg [water])
C_i	=	COPC concentration in *i*th plant or animal food item (mg COPC/kg)
C_M	=	COPC concentration in media (mg/kg [soil, sediment], or mg/L [water])

Equation 5-3 estimates a COPC concentration in an invertebrate, phytoplankton, and rooted aquatic plant to evaluate dose ingested to the measurement receptor. Calculation of COPC concentrations in media is further discussed in Chapter 3 and Appendix B. Media-to-receptor *BCF*s are receptor- and media-specific, and values along with supporting discussion are provided in Appendix C. Appendix F provides specific equations and supporting discussion for calculating COPC concentrations in plant and animal food items.

Equilibrium Partitioning (EqP) Approach

When adequate site-specific characterization data is available, specifically organic carbon fraction data for soil and sediment, the permitting authority may elect in some cases to allow the calculation of COPC concentrations in soil invertebrate (Connell and Markwell 1990) or sediment invertebrate (U.S. EPA 1993q) using the equilibrium partitioning (EqP) approach. However, the EqP approach is not prefered over use of measured BCF values multiplied by the COPC concentration in the media (i.e., sediment or soil), following the approach previously discussed.

The EqP approach utilizes the correlation of the concentrations of nonionic organic compounds in sediment, on an organic carbon basis, to their concentrations in the interstitial water, to determine the observed biological effects on sediment invertebrate (U.S. EPA 1993q). The EqP approach is only applicable for (1) hydrophobic nonionic organic compounds, (2) soil- and sediment-invertebrates, and (3) COPCs with empirical water bioconcentration factors (U.S. EPA 1993q). Also, the EqP approach assumes that the partitioning of the compound in sediment organic carbon and interstitial water are in equilibrium, and the sediment—interstitial water equilibrium system provides the same exposure as a water-only exposure (U.S. EPA 1993q).

To calculate the COPC concentration in an invertebrate using the EqP approach, the soil or sediment interstitial water concentration should be multiplied by the *BCF* determined from a water exposure for a benthic invertebrate:

$$C_I = C_{IW} \cdot BCF_{WI} \qquad \text{Equation 5-4}$$

where

C_I	=	COPC concentration in soil or benthic invertebrate (mg/kg)
C_{IW}	=	COPC concentration in soil or sediment interstitial water (mg/L)
BCF_{WI}	=	Bioconcentration factor for water-to-invertebrate (L/kg)

Equation 5-5 is used to calculate the COPC concentration in soil or sediment interstitial water for this approach:

$$C_{IW} = \frac{C_M}{f_{oc} \cdot K_{oc}} \qquad \text{Equation 5-5}$$

where

C_{IW}	=	COPC concentration in soil or sediment interstitial water (mg/L)
C_M	=	COPC concentration in media (mg/kg [soil, sediment])
f_{oc}	=	Fraction of organic carbon in soil or sediment (unitless)
K_{oc}	=	Organic carbon partitioning coefficient (L/kg)

5.3.2.2 COPC Concentration in Terrestrial Plants

The COPC concentration in terrestrial plants (C_{TP}) is calculated by summing the plant concentration due to direct deposition (*Pd*), air-to-plant transfer (*Pv*), and root uptake (*Pr*). Equation 5-6 should be used to compute a COPC concentration in terrestrial plants:

$$C_{TP} = Pd + Pv + Pr \qquad \text{Equation 5-6}$$

where

C_{TP}	=	COPC concentration in terrestrial plants (mg COPC/kg WW)
Pd	=	COPC concentration in plant due to to direct deposition (mg/kg WW)
Pv	=	COPC concentration in plant due to air-to-plant transfer (mg/kg WW)
Pr	=	COPC concentration in plant due to root uptake (mg/kg WW)

Calculation of *Pd*, *Pv*, and *Pr* is presented in Chapter 3 and Appendix B. Calculation of C_{TP} is further discussed in Appendix F.

5.3.2.3 COPC Concentration in Fish

The COPC concentration in fish is calculated by multiplying a COPC-specific *BCF* and trophic level-specific *FCM* by the dissolved water concentration, as follows:

$$C_F = BCF \cdot FCM \cdot C_{dw} \qquad \text{Equation 5-7}$$

where

C_F	=	COPC concentration in fish (mg/kg)
BCF	=	Bioconcentration factor for water-to-fish (L/kg)
FCM	=	Food-chain multiplier (unitless)
C_{dw}	=	Dissolved phase water concentration (mg/L)

The COPC concentration in fish is calculated using dissolved phase water concentrations, since bioconcentration, or estimated bioaccumulation, values are typically derived from studies based on dissolved phase water concentrations. The *FCM* used to calculate a COPC concentration in fish should be appropriate for the trophic level of the fish ingested by a measurement receptor. Development of *FCM* values is discussed in the following subsection, and actual recommended values are provided in Table 5-2. The dissolved phase water concentration is calculated as discussed in Chapter 3 and Appendix B. Values for bioconcentration factors for water-to-fish, and discussion on their determination, can be found in Appendix C. Calculation of C_F is further discussed in Appendix F.

Food-Chain Multipliers

*FCM*s presented in Table 5-2 were adopted directly from U.S. EPA (1995k), which determined them for K_{ow} values ranging from 3.5 through 9.0 using the Gobas (1993) model. U.S. EPA determined *FCM*s to develop water criteria protective to wildlife of the Great Lakes (U.S. EPA 1995j). As presented in Equation 5-8, U.S. EPA (1995k) calculated trophic level specific *FCM*s (see Table 5-2) utilizing *BAF* values obtained from the Gobas (1993) model and compound specific K_{ow} values.

$$FCM = \frac{BAF_l}{K_{ow}} \qquad \text{Equation 5-8}$$

where

FCM	=	Food-chain multiplier (unitless)
BAF_l	=	Bioaccumulation factor reported on a lipid-normalized basis using the freely dissolved concentration of a chemical in the water (L/kg)
K_{ow}	=	Octanol-water partition coefficient (L/kg)

BAF values predicted using the Gobas (1993) model were based on chemical concentrations in both the water column and surface sediment. Bioaccumulation values for fish were determined from the rate of chemical uptake, the rate of chemical depuration (including excretion), metabolism, and dilution due to growth. As reported in U.S.. EPA (1995k), data on physicochemical parameters and species characteristics reported by Oliver and Niimi (1988), Flint (1986), and Gobas (1993) were used.

For each K_{ow} value, the Gobas (1993) model reported correlating BAF_l values specific to each organism in the food web. U.S. EPA (1995k) determined trophic level-specific *FCM*s by calculating the geometric mean of the *FCM* for each organism in each respective trophic level. The *FCM*s were developed assuming no metabolism of a compound. Thus, for compounds where metabolism may occur (i.e., some PAHs), the COPC concentration in fish ingested by a measurement receptor may be overestimated. This information should be noted as an uncertainty in risk characterization. It should also be noted that the *FCM* values presented in Table 5-2 were developed using K_{ow} values reported in U.S. EPA (1995k); which may differ from K_{ow} values specified in Appendix A-2 of this guidance.

Using the U.S. EPA (1995k) assumption that a compound's log K_{ow} value approximates its BCF_l, Equation 5-8 for determining *FCM* values can also be expressed as follows:

$$FCM = \frac{BAF_l}{BCF_l} \qquad \text{Equation 5-9}$$

where

FCM	=	Food-chain multiplier (unitless)
BAF_l	=	Bioaccumulation factor reported on a lipid-normalized basis using the freely dissolved concentration of a chemical in the water (L/kg)
BCF_l	=	Bioconcentration factor reported on a lipid-normalized basis using the freely dissolved concentration of a chemical in the water (L/kg)

Equation 5-9 can also be written to demonstrate the relation of a *BCF* multiplied by a *FCM* to estimate a *BAF*, as shown in the following equation:

$$BAF = BCF \cdot FCM \qquad \text{Equation 5-10}$$

where

BAF	=	Bioaccumulation factor (L/kg)
BCF	=	Bioconcentration factor (L/kg)
FCM	=	Trophic level-specific food-chain multiplier (unitless)

*FCM*s are specified for use in this guidance to model a COPC concentration in fish, and also mammalian and bird food items, that are ingested by a measurement receptor. The *BCF-FCM* approach accounts for the uptake or bioaccumulation of COPCs into organisms, typically represented in equations as a *BAF* (U.S. EPA 1995j). The availability of data allows the *BCF-FCM* approach to be more consistently applied across class-specific guilds within food webs being evaluated.

U.S. EPA OSW recognizes the limitations and uncertainties of applying *FCM*s derived from aquatic food web data to terrestrial receptors, as well as all top level consumers, whether their food is chiefly aquatic or not. However, the *BCF-FCM* approach is recommended in this guidance because (1) evaluation of multiple food chain exposure pathways is typically required to estimate risk to multiple mammalian and avian guilds in several food webs, (2) screening level risk assessment results are intended to support develoment of permits and focus risk management efforts, rather than as a final point of departure for further evaluation, and (3) U.S. EPA OSW is aware of no other applicable multipathway approaches for consistently and reproducibly estimating COPC concentrations in prey ingested by upper-trophic-level ecological receptors, considering current data limitations. Therefore, U.S. EPA OSW believes the *BCF-FCM* approach is the best available quantitative method for estimating COPC concentrations in upper trophic level food items ingested by measurement receptors, considering data availabilty and the objectives inherent to a screening level risk assessment.

TABLE 5-2

FOOD-CHAIN MULTIPLIERS

Log K_{ow}	Trophic Level of Consumer		
	2	3	4
2.0	1.0	1.0	1.0
2.5	1.0	1.0	1.0
3.0	1.0	1.0	1.0
3.1	1.0	1.0	1.0
3.2	1.0	1.0	1.0
3.3	1.0	1.1	1.0
3.4	1.0	1.1	1.0
3.5	1.0	1.1	1.0
3.6	1.0	1.1	1.0
3.7	1.0	1.1	1.0
3.8	1.0	1.2	1.0
3.9	1.0	1.2	1.1
4.0	1.0	1.3	1.1
4.1	1.0	1.3	1.1
4.2	1.0	1.4	1.1
4.3	1.0	1.5	12
4.4	1.0	1.6	1.2
4.5	1.0	1.8	1.3
4.6	1.0	2.0	1.5
4.7	1.0	2.2	1.6
4.8	1.0	2.5	1.9
4.9	1.0	2.8	2.2
5.0	1.0	3.2	2.6
5.1	1.0	3.6	3.2
5.2	1.0	4.2	3.9
5.3	1.0	4.8	4.7
5.4	1.0	5.5	5.8
5.5	1.0	6.3	7.1
5.6	1.0	7.1	8.6

TABLE 5-2

FOOD-CHAIN MULTIPLIERS

Log K_{ow}	Trophic Level of Consumer		
	2	3	4
5.7	1.0	8.0	10
5.8	1.0	8.8	12
5.9	1.0	9.7	14
6.0	1.0	11	16
6.1	1.0	11	18
6.2	1.0	12	20
6.3	1.0	13	22
6.4	1.0	13	23
6.5	1.0	14	25
6.6	1.0	14	26
6.7	1.0	14	26
6.8	1.0	14	27
6.9	1.0	14	27
7.0	1.0	14	26
7.1	1.0	14	25
7.2	1.0	14	24
7.3	1.0	13	23
7.4	1.0	13	21
7.5	1.0	13	19
7.6	1.0	12	17
7.7	1.0	11	14
7.8	1.0	10	12
7.9	1.0	9.2	9.8
8.0	1.0	8.2	7.8
8.1	1.0	7.3	6.0
8.2	1.0	6.4	4.5
8.3	1.0	5.5	3.3
8.4	1.0	4.7	2.4
8.5	1.0	3.9	1.7
8.6	1.0	3.3	1.1

TABLE 5-2

FOOD-CHAIN MULTIPLIERS

Log K_{ow}	Trophic Level of Consumer		
	2	3	4
8.7	1.0	2.7	0.78
8.8	1.0	2.2	0.52
8.9	1.0	1.8	0.35
9.0	1.0	1.5	0.23

Source: U.S. EPA. 1995k. "Great Lakes Water Quality Initiative Technical Support Document for the Procedure to Determine Bioaccumulation factors." EPA-820-B-95-005. Office of Water. Washington, D.C. March.

5.3.2.4 COPC Concentration in Mammals, Birds, Amphibians, and Reptiles

The COPC concentration in mammals and birds, as food items ingested by measurement receptors, are estimated using equations specific to each guild (i.e., herbivores, omnivores, and carnivores), and based on the plant and animal food items, and media ingested. Similar to calculating the COPC concentration in fish, a *BCF-FCM* approach is used to account for bioaccumulation. However, the contribution of COPC concentrations from each food item ingested must be accounted for directly for wildlife, whereas, the derivation of *BCF-FCM* values already accounts for the COPC contributions from all pathways for fish. Also for wildlife, a ratio of *FCM*s is applied to each animal food item ingested to account for the increase in COPC concentration occurring between the trophic level of the prey item (TLn) and the trophic level of the omnivore (TL3) or carnivore (TL4).

General equations for estimating COPC concentrations of food items in each guild, including use of a *FCM* ratio to estimate biomagnification, are described in the following subsections using mammals and birds as examples. Specific equations and discussion of associated parameters are provided in Appendix F. It should be noted that due to limited availabilty of biotransfer and toxicity data for reptiles and amphibians, the equations in the following subsections and in Appendix F have not been specifically described for use to model exposure to these receptors. However, if site-specific conditions and data warrant evaluation of reptiles and amphibians, the permitting authority may elect to utilize the same generic equations presented.

STEP 1: SCREENING-LEVEL PROBLEM FORMULATION AND ECOLOGICAL EFFECTS EVALUATION

OVERVIEW

The screening-level problem formulation and ecological effects evaluation is part of the initial ecological risk screening assessment. For this initial step, it is likely that site-specific information for determining the nature and extent of contamination and for characterizing ecological receptors at the site is limited. This step includes all the functions of problem formulation (more fully described in Steps 3 and 4) and ecological effects analysis, but on a screening level. The results of this step will be used in conjunction with exposure estimates in the preliminary risk calculation in Step 2.

1.1 INTRODUCTION

Step 1 is the screening-level problem formulation process and ecological effects evaluation (Highlight 1-1 defines screening-level risk assessments). Consultation with the BTAG is recommended at this stage. How to brief the BTAG on the setting, history, and ecology of a site is described in *ECO Update Volume 1, Number 5* (U.S. EPA, 1992d). Section 1.2 describes the screening-level problem formulation, and Section 1.3 describes the screening-level ecological effects evaluation. Section 1.4 summarizes this step.

1.2 SCREENING-LEVEL PROBLEM FORMULATION

For the screening-level problem formulation, the risk assessor develops a conceptual model for the site that addresses five issues:

(1) Environmental setting and contaminants known or suspected to exist at the site (Section 1.2.1);

(2) Contaminant fate and transport mechanisms that might exist at the site (Section 1.2.2);

(3) The mechanisms of ecotoxicity associated with contaminants and likely categories of receptors that could be affected (Section 1.2.3);

(4) What complete exposure pathways might exist at the site (a complete exposure pathway is one in which the chemical can be traced or expected to travel from the source to a receptor that can be affected by the chemical) (Section 1.2.4); and

(5) Selection of endpoints to screen for ecological risk (Section 1.2.5).

HIGHLIGHT 1-1
Screening-level Risk Assessments

Screening-level risk assessments are simplified risk assessments that can be conducted with limited data by assuming values for parameters for which data are lacking. At the screening level, it is important to minimize the chances of concluding that there is no risk when in fact a risk exists. Thus, for exposure and toxicity parameters for which site-specific information is lacking, assumed values should consistently be biased in the direction of overestimating risk. This ensures that sites that might pose an ecological risk are studied further. Without this bias, a screening evaluation could not provide a defensible conclusion that negligible ecological risk exists or that certain contaminants and exposure pathways can be eliminated from consideration.

1.2.1 Environmental Setting and Contaminants at the Site

To begin the screening-level problem formulation, there must be at least a rudimentary knowledge of the potential environmental setting and chemical contamination at the site. The first step is to compile information from the site history and from reports related to the site, including the Preliminary Assessment (PA) or Site Investigation (SI). The second step is to use the environmental checklist presented in *Representative Sampling Guidance Document, Volume 3: Ecological* (U.S. EPA, 1997; see Appendix B) to begin characterizing the site for problem formulation. Key questions addressed by the checklist include:

- What are the on- and off-site land uses (e.g., industrial, residential, or undeveloped; current and future)?
- What type of facility existed or exists at the site?
- What are the suspected contaminants at the site?
- What is the environmental setting, including natural areas (e.g., upland forest, on-site stream, nearby wildlife refuge) as well as disturbed/man-made areas (e.g., waste lagoons)?
- Which habitats present on site are potentially contaminated or otherwise disturbed?
- Has contamination migrated from source areas and resulted in "off-site" impacts or the threat of impacts in addition to on-site threats or impacts?

These questions should be answered using the site reports, maps (e.g, U.S. Geological Survey, National Wetlands Inventory), available aerial photographs, communication with appropriate agencies (e.g., U.S. Fish and Wildlife Service, National Oceanic and Atmospheric Administration, State Natural Heritage Programs), and a site visit. Activities that should be conducted during the site visit include:

- Note the layout and topography of the site;
- Note and describe any water bodies and wetlands;
- Identify and map evidence indicating contamination or potential contamination (e.g., areas of no vegetation, runoff gullies to surface waters);
- Describe existing aquatic, terrestrial, and wetland ecological habitat types (e.g., forest, old field), and estimate the area covered by those habitats;
- Note any potentially sensitive environments (see Section 1.2.3 for examples of sensitive environments);
- Describe and, if possible, map soil and water types, land uses, and the dominant vegetation species present; and
- Record any observations of animal species or sign of a species.

Mapping can be useful in establishing a "picture" of the site to assist in problem formulation. The completed checklist (U.S. EPA, 1997) will provide information regarding habitats and species potentially or actually present on site, potential contaminant migration pathways, exposure pathways, and the potential for non-chemical stresses at the site.

After finishing the checklist, it might be possible to determine that present or future ecological impacts are negligible because complete exposure pathways do not exist and could not exist in the future. Many Superfund sites are located in highly industrialized areas where there could be few if any ecological receptors or where site-related impacts might be indistinguishable from non-site-related impacts (see Highlight 1-2). For such sites, remediation to reduce ecological risks might not be needed. However, all sites should be evaluated by qualified personnel to determine whether this conclusion is appropriate.

Other Superfund sites are located in less disturbed areas with protected or sensitive environments that could be at risk of adverse effects from contaminants from the site. State and federal laws (e.g., the Clean Water Act, the Endangered Species Act) designate certain types of environments as requiring protection. Other types of habitats unique to certain areas also could need special consideration in the risk assessment (see Section 1.2.3).

1.2.2 Contaminant Fate and Transport

During problem formulation, pathways for migration of a contaminant (e.g., windblown dust, surface water runoff, erosion) should be identified. These pathways can exhibit a decreasing gradient of contamination with increasing distance from a site. There are exceptions, however, because physical and chemical characteristics of the media also influence contaminant distribution (e.g., the pattern of sediment deposition in streams varies depending on stream flow and bottom characteristics). For the screening-level risk assessment, the highest contaminant concentrations measured on the site should be documented for each medium.

HIGHLIGHT 1-2
Industrial or Urban Settings

Many hazardous waste sites exist in currently or historically industrialized or urbanized areas. In these instances, it can be difficult to distinguish between impacts related to contaminants from a particular site and impacts related to non-contaminant stressors or to contaminants from other sites. However, even in these cases, it could be appropriate to take some remedial actions based on ecological risks. These actions might be limited to source removal or might be more extensive. An ecological risk assessment can assist the risk manager in determining what action, if any, is appropriate.

1.2.3 Ecotoxicity and Potential Receptors

Understanding the toxic mechanism of a contaminant helps to evaluate the importance of potential exposure pathways (see Section 1.2.4) and to focus the selection of assessment endpoints (see Section 1.2.5). Some contaminants, for example, affect primarily vertebrate animals by interfering with organ systems not found in invertebrates or plants (e.g., distal tubules of vertebrate kidneys, vertebrate hormone systems). Other substances might affect primarily certain insect groups (e.g., by interfering with hormones needed for metamorphosis), plants (e.g., herbicides), or other groups of organisms. For substances that affect, for example, reproduction of mammals at much lower environmental exposure levels than they affect other groups of organisms, the screening-level risk assessment can initially focus on exposure pathways and risks to mammals. Example 1-1 illustrates this point using the PCB site example provided in Appendix A. A review of some of the more recent ecological risk and toxicity assessment literature can help identify likely effects of the more common contaminants at Superfund sites.

An experienced biologist or ecologist can determine what plants, animals, and habitats exist or can be expected to exist in the area of the Superfund site. Exhibit 1-1, adapted from the Superfund Hazard Ranking System, is a partial list of types of sensitive environments that could require protection or special consideration. Information obtained for the environmental checklist (Section 1.2.1), existing information and maps, and aerial photographs should be used to identify the presence of sensitive environments on or near a site that might be threatened by contaminants from the site.

EXAMPLE 1-1
Ecotoxicity PCB Site

Some PCBs are reproductive toxins in mammals (Ringer et al., 1972; Aulerich et al., 1985; Wren et al., 1991; Kamrin and Ringer, 1996). When ingested, they induce (i.e., increase concentrations and activity of) enzymes in the liver, which might affect the metabolism of some steroid hormones (Rice and O'Keefe, 1995). Whatever the mechanism of action, several physiological functions that are controlled by steroid hormones can be altered by the exposure of mammals to certain PCBs, and reproduction appears to be the most sensitive endpoint for PCB toxicity in mammals (Rice and O'Keefe, 1995). Given this information, the screening ecological risk assessment should include potential exposure pathways for mammals to PCBs that are reproductive toxins (see Example 1-2).

1.2.4 Complete Exposure Pathways

Evaluating potential exposure pathways is one of the primary tasks of the screening-level ecological characterization of the site. For an exposure pathway to be complete, a contaminant must be able to travel from the source to ecological receptors and to be taken up by the receptors via one or more exposure routes. (Highlight 1-3 defines exposure pathway and exposure route.) Identifying complete exposure pathways prior to a quantitative evaluation of toxicity allows the assessment to focus on only those contaminants that can reach ecological receptors.

Different exposure routes are important for different groups of organisms. For terrestrial animals, three basic exposure routes need to be evaluated: inhalation, ingestion, and dermal absorption. For terrestrial plants, root absorption of contaminants in soils and leaf absorption of contaminantsevaporating from the soil or deposited on the leaves are of concern at Superfund sites. For aquatic animals, direct contact (of water or sediment with the gills or integument) and ingestion of food (and sometimes sediments) should be considered. For aquatic plants, direct contact with water, and sometimes with air or sediments, is of primary concern.

HIGHLIGHT 1-3
Exposure Pathway and Exposure Route

Exposure Pathway: The pathway by which a contaminant travels from a source (e.g., drums, contaminated soils) to receptors. A pathway can involve multiple media (e.g., soil runoff to surface waters and sedimentation, or volatilization to the atmosphere).

Exposure Route: A point of contact/entry of a contaminant from the environment into an organism (e.g., inhalation, ingestion, dermal absorption).

The most likely exposure pathways and exposure routes also are related to the physical and chemical properties of the contaminant (e.g., whether or not the contaminant is bound to a matrix, such as organic carbon). Of the basic exposure routes identified above, more information generally is available to quantify exposure levels for ingestion by terrestrial animals and for direct contact with water or sediments by aquatic organisms than for other exposure routes and receptors. Although other

exposure routes can be important, more assumptions are needed to estimate exposure levels for those routes, and the results are less certain. Professional judgment is needed to determine if evaluating those routes sufficiently improves a risk assessment to warrant the effort.

If an exposure pathway is not complete for a specific contaminant (i.e., ecological receptors cannot be exposed to the contaminant), that exposure pathway does not need to be evaluated further. For example, suppose a contaminant that impairs reproduction in mammals occurs only in soils that are well below the root zone of plants that occur or are expected to occur on a site. Herbivorous mammals would not be exposed to the contaminant through their diets because plants would not be contaminated. Assuming that most soil macroinvertebrates available for ingestion live in the root zone, insectivorous mammals also would be unlikely to be exposed. In this case, a complete exposure pathway for this contaminant for ground-dwelling mammals would not exist, and the contaminant would not pose a significant risk to this group of organisms. Secondary questions might include whether the contaminant is leaching from the soil to ground water that discharges to surface water, thereby posing a risk to the aquatic environment or to terrestrial mammals that drink the water or consume aquatic prey. Example 1-2 illustrates the process of identifying complete exposure pathways based on the hypothetical PCB site described in Appendix A.

1.2.5 Assessment and Measurement Endpoints

For the screening-level ecological risk assessment, assessment endpoints are any adverse effects on ecological receptors, where receptors are plant and animal populations and communities, habitats, and sensitive environments. Adverse effects on populations can be inferred from measures related to impaired reproduction, growth, and survival. Adverse effects on communities can be inferred from changes in community structure or function. Adverse effects on habitats can be inferred from changes in composition and characteristics that reduce the habitats' ability to support plant and animal populations and communities.

Many of the screening ecotoxicity values now available or likely to be available in the future for the Superfund program (see Section 1.3) are based on generic assessment endpoints (e.g., protection of aquatic communities from changes in structure or function) and are assumed to be widely applicable to sites around the United States.

EXHIBIT 1-1
List of Sensitive Environments in the Hazard Ranking System[a]

Critical habitat for Federal designated endangered or threatened species
Marine Sanctuary
National Park
Designated Federal Wilderness Area
Areas identified under the Coastal Zone Management Act
Sensitive areas identified under the National Estuary Program or Near Coastal Waters Program
Critical areas identified under the Clean Lakes Program
National Monument
National Seashore Recreational Area
National Lakeshore Recreational Area
Habitat known to be used by Federal designated or proposed endangered or threatened species
National Preserve
National or State Wildlife Refuge
Unit of Coastal Barrier Resources System
Coastal Barrier (undeveloped)
Federal land designated for protection of natural ecosystems
Administratively Proposed Federal Wilderness Area
Spawning areas critical for the maintenance of fish/shellfish species within river, lake, or coastal tidal waters
Migratory pathways and feeding areas critical for maintenance of anadromous fish species within river reaches or areas in lakes or coastal tidal waters in which the fish spend extended periods of time
Terrestrial areas utilized for breeding by large or dense aggregations of animals
National river reach designated as Recreational
Habitat known to be used by state designated endangered or threatened species
Habitat known to be used by species under review as to its Federal endangered or threatened status
Coastal Barrier (partially developed)
Federally-designated Scenic or Wild River
State land designated for wildlife or game management
State-designated Scenic or Wild River
State-designated Natural Areas
Particular areas, relatively small in size, important to maintenance of unique biotic communities
State-designated areas for protection or maintenance of aquatic life
Wetlands[b]

[a] The categories are listed in groups from those assigned higher factor values to those assigned lower factor values in the Hazard Ranking System (HRS) for listing hazardous waste sites on the National Priorities List (U.S. EPA, 1990b). See *Federal Register*, Vol. 55, pp. 51624 and 51648 for additional information regarding definitions.

[b] Under the HRS, wetlands are rated on the basis of size. See *Federal Register*, Vol. 55, pp. 51625 and 51662 for additional information.

EXAMPLE 1-2
Complete Exposure Pathways for Mammals PCB Site

Three possible exposure pathways for mammals were evaluated at the PCB Site: inhalation, ingestion through the food chain, and incidental soil/sediment ingestion.

Inhalation. PCBs are not highly volatile, so the inhalation of PCB vapors by mammals would be an essentially incomplete exposure pathway. Inhalation of PCBs adsorbed to soil particles might need consideration in areas with exposed soils, but this site is well vegetated.

Ingestion through the food chain. PCBs tend to bioaccumulate and biomagnify in food chains. PCBs in soils are not taken up by most plants, but are accumulated by soil macroinvertebrates. Thus, in areas without significant soil deposition on the surfaces of plants, mammalian herbivores would not be exposed to PCBs in most of their diet. In contrast, mammalian insectivores, such as shrews, could be exposed to PCBs in most of their diet. For PCBs, the ingestion route for mammals would be essentially incomplete for herbivores but complete for insectivores. For the PCB site, therefore, the ingestion exposure route for a mammalian insectivore (e.g., shrew) would be a complete exposure pathway that should be evaluated.

Incidental soil/sediment ingestion. Mammals can ingest some quantity of soils or sediments incidentally, as they groom their fur or consume plants or animals from the soil. Burrowing mammals are likely to ingest greater quantities of soils during grooming than non-burrowing mammals, and mammals that consume plant roots or soil-dwelling macroinvertebrates are likely to ingest greater quantities of soils attached to the surface of their foods than mammals that consume other foods. The intake of PCBs from incidental ingestion of PCB-contaminated soils is difficult to estimate, but for insectivores that forage at ground level, it is likely to be far less than the intake of PCBs in the diet. For herbivores, the incidental intake of PCBs in soils might be higher than the intake of PCBs in their diet, but still less than the intake of PCBs by mammals feeding on soil macroinvertebrates. Thus, the exposure pathway for ground-dwelling mammalian insectivores remains the exposure pathway that should be evaluated.

1.3 SCREENING-LEVEL ECOLOGICAL EFFECTS EVALUATION

The next step in the screening-level risk assessment is the preliminary ecological effects evaluation and the establishment of contaminant exposure levels that represent conservative thresholds for adverse ecological effects. In this guidance, those conservative thresholds are called screening ecotoxicity values. Physical stresses unrelated to contaminants at the site are not the focus of the risk assessment (see Highlight 1-4), although they can be considered later when evaluating effects of remedial alternatives.

A literature search for studies that quantify toxicity (i.e., exposure-response) is necessary to evaluate the likelihood of toxic effects in different groups of organisms. Appendix C provides a basic introduction to conducting a literature search, but an expert should be consulted to minimize time and costs. The toxicity profile should describe the toxic mechanisms of action for the exposure routes being evaluated and the dose or environmental concentration that causes a specified adverse effect.

For each complete exposure pathway, route, and contaminant, a screening ecotoxicity value should be developed.[1] The U.S. EPA Office of Emergency and Remedial Response has developed screening ecotoxicity values [called ecotox threshold values (U.S. EPA, 1996c)]. The values are for surface waters and sediments, and are based on direct exposures routes only; bioaccumulation and biomagnification in food chains have not been accounted for. The following subsections describe preferred data (Section 1.3.1), dose conversions (Section 1.3.2), and analyzing uncertainty in the values (Section 1.3.3).

1.3.1 Preferred Toxicity Data

Screening ecotoxicity values should represent a no-observed-adverse-effect-level(NOAEL)forlong-term (chronic) exposures to a contaminant. Ecological effects of most concern are those that can impact populations (or higher levels of biological organization). Those include adverse effects on development, reproduction, and survivorship. Community-level effects also can be of concern, but toxicity data on community-level endpoints are limited and might be difficult to extrapolate from one community to another.

HIGHLIGHT 1-4
Non-Chemical Stressors

Ecosystems can be stressed by physical, as well as by chemical, alterations of their environment. For this reason, EPA's (1992a) *Framework for Ecological Risk Assessment* addresses "stressor-response" evaluation to include all types of stress instead of "dose-response" or "exposure-response" evaluation, which implies that the stressor must be a toxic substance.

For Superfund sites, however, the baseline risk assessment addresses risks from hazardous substances released to the environment, not risks from physical alterations of the environment, unless caused indirectly by a hazardous substances (e.g., loss of vegetation from a chemical release leading to serious erosion). This guidance document, therefore, focuses on exposure-response evaluations for toxic substances. Physical destruction of habitat that might be associated with a particular remedy is considered in the Feasibility Study.

When reviewing the literature, one should be aware of the limitations of published information in characterizing actual or probable hazards at a specific site. U.S. EPA discourages reliance on secondary references because study details relevant for determining the applicability of findings to a given site usually are not reported in secondary sources. Only primary literature that has been carefully reviewed by an ecotoxicologist should be used to support a decision. Several considerations and data preferences are summarized in Highlight 1-5 and described more fully below.

NOAELS and LOAELS. For each contaminant for which a complete exposure pathway/route exists, the literature should be reviewed for the lowest exposure level (e.g., concentration in water or in the diet, ingested dose) shown to produce adverse effects (e.g.,reduced growth, impaired reproduction, increased mortality) in a potential receptor species. This value is called a lowest-observed-adverse-

[1] It is possible to conduct a screening risk assessment with limited information and conservative assumptions. If site-specific information is too limited, however, the risk assessment is almost certain to move into Steps 3 through 7, which require field-collected data. The more complete the initial information, the better the decision that can be made at this preliminary stage.

effect-level or LOAEL. For those contaminants with documented adverse effects, one also should identify the highest exposure level that is a NOAEL. A NOAEL is more appropriate than a LOAEL to use as an screening ecotoxicity value to ensure that risk is not underestimated (see Highlight 1-6). However, NOAELs currently are not available for many groups of organisms and many chemicals. When a LOAEL value, but not a NOAEL value, is available from the literature, a standard practice is to multiply the LOAEL by 0.1 and to use the product as the screening ecotoxicity value. Support for this practice comes from a data review indicating that 96 percent of chemicals included in the review had LOAEL/NOAEL ratios of five or less, and that all were ten or less (Dourson and Stara, 1983).

Exposure duration. Data from studies of chronic exposure are preferable to data from medium-term (subchronic), short-term (acute), or single-exposure studies because exposures at Superfund remedial sites usually are long-term. Literature reviews by McNamara (1976) and Weil and McCollister (1963) indicate that [2] chronic NOAELs can be lower than subchronic (90-day duration for rats) NOAELs by up to a factor of ten[2].

HIGHLIGHT 1-5
Data Hierarchy for Deriving Screening Ecotoxicity Values

To develop a chronic NOAEL for a screening ecotoxicity value from existing literature, the following data hierarchy minimizes extrapolations and uncertainties in the value:

- A NOAEL is preferred to a LOAEL, which is preferred to an LC_{50} or an EC_{50}.
- Long-term (chronic) studies are preferred to medium-term (subchronic) studies, which are preferred to short-term (acute) studies.
- If exposure at the site is by ingestion, dietary studies are preferred to gavage studies, which are preferred to non-ingestion routes of exposure. Similarly, if exposure at the site is dermal, dermal studies are preferred to studies using other exposure routes.

Exposure route. The exposure route and medium used in the toxicity study should be comparable to the exposure route in the risk assessment. For example, data from studies where exposure is by gavage generally are not preferred for estimating dietary concentrations that could produce adverse effects, because the rate at which the substance is absorbed from the gastrointestinal tract usually is greater following gavage than following dietary administration. Similarly, intravenous injection of a substance results in "instantaneous absorption" and does not allow the substance to first pass through the liver, as it would following dietary exposure. If it is necessary to attempt to extrapolate toxicity test results from one route of exposure to another, the extrapolation should be performed or reviewed by a toxicologist experienced in route-to-route extrapolations for the class of animals at issue.

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[2] The literature reviews of McNamara (1976) and Weil and McCollister (1963) included both rodent and non-rodent species. The duration of the subchronic exposure usually was 90 days, but ranged from 30 to 210 days. A wide variety of endpoints and criteria for adverse effects were included in these reviews. Despite this variation in the original studies, their findings provide a general indication of the ratio between subchronic to chronic NOAELs for effects other than cancer and reproductive effects. For some chemicals, chronic dosing resulted in increased chemical tolerance. For over 50 percent of the compounds tested, the chronic NOAEL was less than the 90-day NOAEL by a factor of 2 or less. However, in a few cases, the chronic NOAEL was up to a factor of 10 less than the subchronic NOAEL (U.S. EPA, 1993e).

Field versus laboratory. Most toxicity studies evaluate effects of a single contaminant on a single species under controlled laboratory conditions. Results from these studies might not be directly applicable to the field, where organisms typically are exposed to more than one contaminant in environmental situations that are not comparable to a laboratory setting and where genetic composition of the population can be more heterogeneous than that of organisms bred for laboratory use. In addition, the bioavailability of a contaminant might be different at a site than in a laboratory toxicity test. In a field situation, organisms also will be subject to other environmental variables, such as unusual weather conditions, infectious diseases, and food shortages. These variables can have either positive or negative effects on the organism's response to a toxic contaminant that only a site-specific field study would be able to evaluate. Moreover, single-species toxicity tests seldom provide information regarding toxicant-related changes in community interactions (e.g., behavioral changes in prey species that make them more susceptible to predation).

1.3.2 Dose Conversions

For some data reported in the literature, conversions are necessary to allow the data to be used for species other than those tested or for measures of exposure other than those reported. Many doses in laboratory studies are reported in terms of concentration in the diet (e.g., mg contaminant/kg diet or ppm in the diet). Dietary concentrations can be converted to dose (e.g., mg contaminant/kg body weight/day) for comparison with estimated contaminant intake levels in the receptor species.

When converting doses, it is important to identify whether weights are measured as wet or dry weights. Usually, body weights are reported on a wet-weight, not dry-weight basis. Concentration of the contaminant in the diet might be reported on a wet- or dry-weight basis.

HIGHLIGHT 1-6
NOAEL Preferred to LOAEL

Because the NOAEL and LOAEL are estimated by hypothesis testing (i.e., by comparing the response level of a test group to the response level of a control group for a statistically significant difference), the actual proportion of the test animals showing the adverse response at an identified LOAEL depends on sample size, variability of the response, and the dose interval. LOAELs, and even NOAELs, can represent a 30 percent or higher effect level for the minimum sample sizes recommended for standard test protocols. For this reason, U.S. EPA recommends that the more conservative NOAELs, instead of LOAELs, are used to determine a screening exposure level that is unlikely to adversely impact populations. If dose-response data are available, a site-specific low-effect level may be determined.

Ingestion rates and body weights for a test species often are reported in a toxicity study or can be obtained from other literature sources (e.g., U.S. EPA, 1993a,b). For extrapolations between animal species with different metabolic rates as well as dietary composition, consult U.S. EPA 1992e and 1996b.

1.3.3 Uncertainty Assessment

Professional judgment is needed to determine the uncertainty associated with information taken from the literature and any extrapolations used in developing a screening ecotoxicity value. The risk assessor

should be consistently conservative in selecting literature values and describe the limitations of using those values in the context of a particular site. Consideration of the study design, endpoints, and other factors are important in determining the utility of toxicity data in the screening-level risk assessment. All of those factors should be addressed in a brief evaluation of uncertainties prior to the screening-level risk calculation.

1.4 SUMMARY

At the conclusion of the screening-level problem formulation and ecological effects evaluation, the following information should have been compiled:

- Environmental setting and contaminants known or suspected to exist at the site and the maximum concentrations present (for each medium);

- Contaminant fate and transport mechanisms that might exist at the site;
  The mechanisms of ecotoxicity associated with contaminants and likely categories of receptors that could be affected;

- The complete exposure pathways that might exist at the site from contaminant sources to receptors that could be affected; and

- Screening ecotoxicity values equivalent to chronic NOAELs based on conservative assumptions.

For the screening-level ecological risk assessment, assessment endpoints will include any likely adverse ecological effects on receptors for which exposure pathways are complete, as determined from the information listed above. Measurement endpoints will be based on the available literature regarding mechanisms of toxicity and will be used to establish the screening ecotoxicity values. Those values will be used with estimated exposure levels to screen for ecological risks, as described in Step 2.

STEP 2: SCREENING-LEVEL EXPOSURE ESTIMATE AND RISK CALCULATION

OVERVIEW

The screening-level exposure estimate and risk calculation comprise the second step in the ecological risk screening for a site. Risk is estimated by comparing maximum documented exposure concentrations with the ecotoxicity screening values from Step 1. At the conclusion of Step 2, the risk manager and risk assessment team will decide that either the screening-level ecological risk assessment is adequate to determine that ecological threats are negligible, or the process should continue to a more detailed ecological risk assessment (Steps 3 through 7). If the process continues, the screening-level assessment serves to identify exposure pathways and preliminary contaminants of concern for the baseline risk assessment by eliminating those contaminants and exposure pathways that pose negligible risks.

2.1 INTRODUCTION

This step includes estimating exposure levels and screening for ecological risks as the last two phases of the screening-level ecological risk assessment. The process concludes with a SMDP at which it is determined that: (1) ecological threats are negligible; (2) the ecological risk assessment should continue to determine whether a risk exists; or (3) there is a potential for adverse ecological effects, and a more detailed ecological risk assessment, incorporating more site-specific information, is needed.

Section 2.2 describes the screening-level exposure assessment, focusing on the complete exposure pathways identified in Step 1. Section 2.3 describes the risk calculation process, including estimating a hazard quotient, documenting the uncertainties in the quotient, and summarizing the overall confidence in the screening-level ecological risk assessment. Section 2.4 describes the SMDP that concludes Step 2.

2.2 SCREENING-LEVEL EXPOSURE ESTIMATES

To estimate exposures for the screening-level ecological risk calculation, on-site contaminant levels and general information on the types of biological receptors that might be exposed should be known from Step 1. Only complete exposure pathways should be evaluated. For these, the highest measured or estimated on-site contaminant concentration for each environmental medium should be used to estimate exposures. This should ensure that potential ecological threats are not missed.

2.2.1 Exposure Parameters

For parameters needed to estimate exposures for which sound site-specific information is lacking or difficult to develop, conservative assumptions should be used at this screening level. Examples of conservative assumptions are listed below and described in the following paragraphs:

- Area-use factor 100 percent (factor related to home range and population density; see Highlight2-1);
- Bioavailability 100 percent;
- Life stage most sensitive life stage;
- Body weight and food ingestion rate minimum body weight to maximum ingestion rate; and
- Dietary composition 100 percent of diet consists of the most contaminated dietary component.

HIGHLIGHT 2-1
Area-use Factor

An animal's area-use factor can be defined as the ratio of the area of contamination (or the site area under investigation) to the area used by the animal, e.g., its home range, breeding range, or feeding/foraging range. To ensure that ecological risks are not underestimated, the highest density and smallest area used by each animal should be assumed. This allows the maximum number of animals to be exposed to site contaminants and makes it more likely that "hot spots" (i.e., areas of unusually high contamination levels) will be significant proportions of an individual animal's home range.

Area-use factor. For the screening level exposure estimate for terrestrial animals, assume that the home range of one or more animals is entirely within the contaminated area, and thus the animals are exposed 100 percent of the time. This is a conservative assumption and, as an assumption, is only applicable to the screening-level phase of the risk assessment. Species- and site-specific home range information would be needed later, in Step 6, to estimate more accurately the percentage of time an animal would use a contaminated area. Also evaluate the possibility that some species might actually focus their activities in contaminated areas of the site. For example, if contamination has reduced emergent vegetation in a pond, the pond might be more heavily used for feeding by waterfowl than uncontaminated ponds with little open water.

Bioavailability. For the screening-level exposure estimate, in the absence of site-specific information, assume that the bioavailability of contaminants at the site is 100 percent. For example, at the screening-level, lead would be assumed to be 100 percent bioavailable to mammals. While some literature indicates that mammals absorb approximately 10 percent of ingested lead, absorption efficiency can be higher, up to about 60 percent, because dietary factors such as fasting, and calcium and phosphate content of the diet, can affect the absorption rate (Kenzaburo, 1986). Because few species have been tested for bioavailability, and because Steps 3 through 6 provide an opportunity for this issue to be addressed specifically, the most conservative assumption is appropriate for this step.

Life stage. For the screening-level assessment, assume that the most sensitive life stages are present. If an early life stage is the most sensitive, the population should be assumed to include or to be in that life stage. For vertebrate populations, it is likely that most of the population is not in the most sensitive life stage most of the time. However, for many invertebrate species, the entire population can be at an early stage of development during certain seasons.

Body weight and food ingestion rates. Estimates of body weight and food ingestion rates of the receptor animals also should be made conservatively to maximize the dose (intake of contaminants) on a body-weight basis and to avoid understating risk, although uncertainties in these factors are far less than the uncertainties associated with the environmental contaminant concentrations. U.S. EPA's *Wildlife Exposure Factors Handbook* (U.S. EPA, 1993a,b) is a good source or reference to sources of this information.

Bioaccumulation. Bioaccumulation values obtained from a literature search can be used to estimate contaminant accumulation and food-chain transfer at a Superfund site at the screening stage. Because many environmental factors influence the degree of bioaccumulation, sometimes by several orders of magnitude, the most conservative (i.e., highest) bioaccumulation factor (BAF) reported in the literature should be used in the absence of site-specific information.

Dietary composition. For species that feed on more than one type of food, the screening-level assumption should be that the diet is composed entirely of whichever type of food is most contaminated. For example, if some foods (e.g., insects) are likely to be more contaminated than other foods (e.g., seeds and fruits) typical in the diet of a receptor species, assume that the receptor species feeds exclusively on the more contaminated type of food. Again, EPA's *Wildlife Exposure Factors Handbook* (U.S. EPA, 1993a,b) is a good source or reference to sources of this information.

2.2.2 Uncertainty Assessment

Professional judgment is needed to determine the uncertainty associated with information taken from the literature and any extrapolations used in developing a parameter to estimate exposures. All assumptions used to estimate exposures should be stated, including some description of the degree of bias possible in each. Where literature values are used, an indication of the range of values that could be considered appropriate also should be indicated.

2.3 SCREENING-LEVEL RISK CALCULATION

A quantitative screening-level risk can be estimated using the exposure estimates developed according to Section 2.2 and the screening ecotoxicity values developed according to Section 1.3. For the screening-level risk calculation, the hazard quotient approach, which compares point estimates of screening ecotoxicity values and exposure values, is adequate to estimate risk. As described in Section 1.3, a screening ecotoxicity value should be equivalent to a documented and/or best conservatively estimated chronic NOAEL. Thus, for each contaminant and environmental medium, the hazard quotient can be expressed as the ratio of a potential exposure level to the NOAEL:
where:

$$HQ = \frac{Dose}{NOAEL} \quad or \quad HQ = \frac{EEC}{NOAEL}$$

HQ = hazard quotient;

Dose = estimated contaminant intake at the site (e.g., mg contaminant/kg body weight per day);

EEC = estimated environmental concentration at the site (e.g., mg contaminant/L water, mg contaminant/kg soil, mg contaminant/kg food); and

NOAEL = no-observed-adverse-effects-level (in units that match the dose or EEC).

An HQ less than one (unity) indicates that the contaminant alone is unlikely to cause adverse ecological effects. If multiple contaminants of potential ecological concern exist at the site, it might be appropriate to sum the HQs for receptors that could be simultaneously exposed to the contaminants that produce effects by the same toxic mechanism (U.S. EPA, 1986a). The sum of the HQs is called a hazard index (HI); (see Highlight 2-2). An HI less than one indicates that the group of contaminants is unlikely to cause adverse ecological effects. An HQ or HI less than one does not indicate the absence of ecological risk; rather, it should be interpreted based on the severity of the effect reported and the magnitude of the calculated quotient. As certainty in the exposure concentrations and the NOAEL increase, there is greater confidence in the predictive value of the hazard quotient model, and unity (HQ = 1) becomes a more certain pass/fail decision point.

HIGHLIGHT 2-2
Hazard Index (HI) Calculation

For contaminants that produce adverse effects by the same toxic mechanism:

Hazard Index = $EEC_1/NOAEL_1 + EEC_2/NOAEL_2 + \ldots + EEC_i/NOAEL_i$

where:

EEC_i = estimated environmental concentration for the i^{th} contaminant; and

NOAELi = NOAEL for the i^{th} contaminant (expressed either as a dose or environmental concentration).

The EEC and the NOAEL are expressed in the same units and represent the same exposure period (e.g., chronic). Dose could be substituted for EEC throughout provided the NOAEL is expressed as a dose.

The screening-level risk calculation is a conservative estimate to ensure that potential ecological threats are not overlooked. The calculation is used to document a decision about whether or not there is a negligible potential for ecological impacts, based on the information available at this stage. If the potential for ecological impacts exists, this calculation can be used to eliminate the negligible-risk combinations of contaminants and exposure pathways from further consideration.

If the screening-level risk assessment indicates that adverse ecological effects are possible at environmental concentrations below standard quantitation limits, a "non detect" based on those limits cannot be used to support a "no risk" decision. Instead, the risk assessment team and risk manager should request appropriate detection limits or agree to continue to Steps 3 through 7, where exposure concentrations will be estimated from other information (e.g., fate-and-transport modeling, assumed or 0 estimated values for non-detects).

2.4 SCIENTIFIC/MANAGEMENT DECISION POINT (SMDP)

At the end of Step 2, the lead risk assessor communicates the results of the preliminary ecological risk assessment to the risk manager. The risk manager needs to decide whether the information available is adequate to make a risk management decision and might require technical advice from the ecological risk assessment team to reach a decision. There are only three possible decisions at this point:

(1) There is adequate information to conclude that ecological risks are negligible and therefore no need for remediation on the basis of ecological risk;

(2) The information is not adequate to make a decision at this point, and the ecological risk assessment process will continue to Step 3; or

(3) The information indicates a potential for adverse ecological effects, and a more thorough assessment is warranted.

Note that the SMDP made at the end of the screening-level risk calculation will not set a preliminary cleanup goal. Screening ecotoxicity values are derived to avoid underestimating risk. Requiring a cleanup based solely on those values would not be technically defensible.

The risk manager should document both the decision and the basis for it. If the risk characterization supports the first decision (i.e., negligible risk), the ecological risk assessment process ends here with appropriate documentation to support the decision. The documentation should include all analyses and references used in the assessment, including a discussion of the uncertainties associated with the HQ and HI estimates.

For assessments that proceed to Step 3, the screening-level analysis in Step 2 can indicate and justify which contaminants and exposure pathways can be eliminated from further assessment because they are unlikely to pose a substantive risk. (If new contaminants are discovered or contaminants are found at higher concentrations later in the site investigation, those contaminants might need to be added to the ecological risk assessment at that time.)

U.S. EPA must be confident that the SMDP made after completion of this calculation will protect the ecological components of the environment. The decision to continue beyond the screening-level risk calculation does not indicate whether remediation is necessary at the site. That decision will be made in Step 8 of the process.

2.5 SUMMARY

At the conclusion of the exposure estimate and screening-level risk calculation step, the following information should have been compiled:

(1) Exposure estimates based on conservative assumptions and maximum concentrations present; and

(2) Hazard quotients (or hazard indices) indicating which, if any, contaminants and exposure pathways might pose ecological threats.

Based on the results of the screening-level ecological risk calculation, the risk manager and lead risk assessor will determine whether or not contaminants from the site pose an ecological threat. If there are sufficient data to determine that ecological threats are negligible, the ecological risk assessment will be complete at this step with a finding of negligible ecological risk. If the data indicate that there is (or might be) a risk of adverse ecological effects, the ecological risk assessment process will continue.

Conservative assumptions have been used for each step of the screening-level ecological risk assessment. Therefore, requiring a cleanup based solely on this information would not be technically defensible. To end the assessment at this stage, the conclusion of negligible ecological risk must be adequately documented and technically defensible. A lack of information on the toxicity of a contaminant or on complete exposure pathways will result in a decision to continue with the ecological risk assessment process (Steps 3 through 7) not a decision to delay the ecological risk assessment until a later date when more information might be available.

STEP 3: BASELINE RISK ASSESSMENT PROBLEM FORMULATION

OVERVIEW

Step 3 of the eight-step process initiates the problem-formulation phase of the baseline ecological risk assessment. Step 3 refines the screening-level problem formulation and, with input from stakeholders and other involved parties, expands on the ecological issues that are of concern at the particular site. In the screening-level assessment, conservative assumptions were used where site-specific information was lacking. In Step 3, the results of the screening assessment and additional site-specific information are used to determine the scope and goals of the baseline ecological risk assessment. Steps 3 through 7 are required only for sites for which the screening-level assessment indicated a need for further ecological risk evaluation.

Problem formulation at Step 3 includes several activities:

- Refining preliminary contaminants of ecological concern;
- Further characterizing ecological effects of contaminants;
- Reviewing and refining information on contaminant fate and transport, complete exposure pathways, and ecosystems potentially at risk;
- Selecting assessment endpoints; and
- Developing a conceptual model with working hypotheses or questions that the site investigation will address.

At the conclusion of Step 3, there is a SMDP, which consists of agreement on four items: the assessment endpoints, the exposure pathways, the risk questions, and conceptual model integrating these components. The products of Step 3 are used to select measurement endpoints and to develop the ecological risk assessment work plan (WP) and sampling and analysis plan (SAP) for the site in Step 4. Steps 3 and 4 are, effectively, the data quality objective (DQO) process for the baseline ecological risk assessment.

3.1 THE PROBLEM-FORMULATION PROCESS

In Step 3, problem formulation establishes the goals, breadth, and focus of the baseline ecological risk assessment. It also establishes the assessment endpoints, or specific ecological values to be protected (U.S. EPA, 1992a). Through Step 3, the questions and issues that need to be addressed in the baseline ecological risk assessment are defined based on potentially complete exposure pathways and ecological effects. A conceptual model of the site is developed that includes questions about the assessment endpoints and the relationship between exposure and effects. Step 3 culminates in an SMDP, which is agreement between the risk manager and risk assessor on the assessment endpoints, exposure pathways, and questions as portrayed in the conceptual model of the site.

The conceptual model, which is completed in Step 4, also will describe the approach, types of data, and analytical tools to be used for the analysis phase of the ecological risk assessment (Step 6). Those components of the conceptual model are formally described in the ecological risk WP and SAP in Step 4 of this eight-step process. If there is not agreement among the risk manager, lead risk assessor, and the other professionals involved with the ecological risk assessment on the initial conceptual model developed in Step 3, the final conceptual model and field study design developed in Step 4 might not resolve the issues that must be considered to manage risks effectively.

The complexity of questions developed during problem formulation does not depend on the size of a site or the magnitude of its contamination. Large areas of contamination can provoke simple questions and, conversely, small sites with numerous contaminants can require a complex series of questions and assessment endpoints. There is no rule that can be applied to gauge the effort needed for an ecological risk assessment based on site size or number of contaminants; each site should be evaluated individually.

At the beginning of Step 3, some basic information should exist for the site. At a minimum, information should be available from the site history, PA, SI, and Steps 1 and 2 of this eight-step process. For large or complex sites, information might be available from earlier site investigations.

It is important to be as complete as possible early in the process so that Steps 3 through 8 need not be repeated. Repeating the selection of assessment endpoints and/or the questions and hypotheses concerning those endpoints is appropriate only if new information indicating new threats becomes available. The SMDP process should prevent having to return to the problem formulation step because of changing opinions on the questions being asked. Repetition of Step 3 should not be confused with the intentional tiering (or phasing) of ecological site investigations at large or complex sites (see Highlight 3-1). The process of problem formulation at complex sites is the same as at more simple sites, but the number, complexity, and/or level of resolution of the questions and hypotheses can be greater at complex sites.

While problem formulation is conceptually simple, in practice it can be a complex and interactive process. Defining the ecological problems to be addressed during the baseline risk assessment involves identifying toxic mechanisms of the contaminants, characterizing potential receptors, and estimating exposure and potential ecological effects. Problem formulation also constitutes the DQO process for the baseline ecological risk assessment (U.S. EPA, 1993c,d).

The remainder of this section describes six activities to be conducted prior to the SMDP for this step: refining preliminary contaminants of ecological concern (Section 3.2); a literature search on the potential ecological effects of the contaminants (Section 3.3); qualitative evaluation of complete exposure pathways and ecosystems potentially at risk (Section 3.4); selecting assessment endpoints (Section 3.5); and developing the conceptual model and establishing risk questions (Section 3.6).

3.2 REFINEMENT OF PRELIMINARY CONTAMINANTS OF CONCERN

The results of the screening-level risk assessment (Steps 1 and 2) should have indicated which contaminants found at the site can be eliminated from further consideration and which should be evaluated further. It is important to realize that contaminants that might pose an ecological risk can be different from those that might pose a human health risk because of differing exposure pathways, sensitivities, and responses to contaminants.

The initial list of contaminants investigated in Steps 1 and 2 included all contaminants identified or suspected to be at the site. During Steps 1 and 2, it is likely that several of the contaminants found at the site were eliminated from further assessment because the risk screen indicated that they posed a negligible ecological risk. Because of the conservative assumptions used during the risk screen, some of the contaminants retained for Step 3 might also pose negligible risk. At this stage, the risk assessor should review the assumptions used (e.g., 100 percent bioavailability) against values reported in the literature (e.g., only up to 60 percent for a particular contaminant), and consider how the HQs would change if more realistic conservative assumptions were used instead (see Section 3.4.1). For those contaminants for which the HQs drop to near or below unity, the lead risk assessor and risk manager should discuss and agree on which can be eliminated from further consideration at this time. The reasons for dropping any contaminants from consideration at this step must be documented in the baseline risk assessment.

HIGHLIGHT 3-1
Tiering an Ecological Risk Assessment

Most ecological risk assessments at Superfund sites are at least a two-tier process. Steps 1 and 2 of this guidance serve as a first, or screening, tier prior to expending a larger effort for a detailed, site-specific ecological risk assessment. The baseline risk assessment may serve as the second tier. Additional tiers could be needed in the baseline risk assessment for large or complex sites where there is a need to sequentially test interdependent hypotheses developed during problem formulation (i.e., evaluating the results of one field assessment before designing a subsequent field study).

While tiering can be an effective way to manage site investigations, multiple sampling phases typically require some resampling of matrices sampled during earlier tiers and increased field-mobilization costs. Thus, in some cases, a multi-tiered ecological risk assessment might cost more than a two-tiered assessment. The benefits of tiering should be weighed against the costs.

Sometimes, new information becomes available that indicates the initial assumptions that screened some contaminants out in Step 2 are no longer valid (e.g., site contaminant levels are higher than originally reported). In this case, contaminants can be placed back on the list of contaminants to be investigated with that justification.

Note that a contaminant should not be eliminated from the list of contaminants to be investigated only because toxicity information is lacking; instead, limited or missing toxicity information must be addressed using best professional judgment and discussed as an uncertainty.

3.3 LITERATURE SEARCH ON KNOWN ECOLOGICAL EFFECTS

The literature search conducted in Step 1 for the screening-level risk assessment might need to be expanded to obtain the information needed for the more detailed problem formulation phase of the baseline ecological risk assessment. The literature search should identify NOAELs, LOAELs, exposure-response functions, and the mechanisms of toxic responses for contaminants for which those data were not collected in Step 1. Appendix C presents a discussion of some of the factors important in conducting a literature search. Several U.S. EPA publications (e.g., U.S. EPA, 1995a,e,g,h) provide a window to original toxicity literature for contaminants often found at Superfund sites. For all retained contaminants, it is important to obtain and review the primary literature.

3.4 CONTAMINANT FATE AND TRANSPORT, ECOSYSTEMS POTENTIALLY AT RISK, AND COMPLETE EXPOSURE PATHWAYS

A preliminary identification of contaminant fate and transport, ecosystems potentially at risk, and complete exposure pathways was conducted in the screening ecological risk assessment. In Step 3, the exposure pathways and the ecosystems associated with the assessment endpoints that were retained by the screening risk assessment are evaluated in more detail. This effort typically involves compiling additional information on:

(1) The environmental fate and transport of the contaminants;

(2) The ecological setting and general flora and fauna of the site (including habitat, potential receptors, etc.); and

(3) The magnitude and extent of contamination, including its spatial and temporal variability relative to the assessment endpoints.

For individual contaminants, it is frequently possible to reduce the number of exposure pathways that need to be evaluated to one or a few "critical exposure pathways" which (1) reflect maximum exposures of receptors within the ecosystem, or (2) constitute exposure pathways to ecological receptors sensitive to the contaminant. The critical exposure pathways influence the selection of assessment endpoints for a particular site. If multiple critical exposure pathways exist, they each should be evaluated, because it is often difficult to predict which pathways could be responsible for the greatest ecological risk.

3.4.1 Contaminant Fate and Transport

Information on how the contaminants will or could be transported or transformed in the environment physically, chemically, and biologically is used to identify the exposure pathways that might lead to significant ecological effects (see Highlight 3-2). Chemically, contaminants can undergo several processes in the environment:

- Degradation,[3]
- Complexation,
- Ionization,
- Precipitation, and/or
- Adsorption.

HIGHLIGHT 3-2
Environmental Fate and Exposure

If a contaminant in an aquatic ecosystem is highly lipophilic (i.e., essentially insoluble in water), it is likely to partition primarily into sediments and not into the water column. Factors such as sediment particle size and organic carbon influence contaminant partitioning; therefore, these attributes should be characterized when sampling sediments. Similar considerations regarding partitioning should be applied to contaminants in soils.

Physically, contaminants might move through the environment by one or more means:

- Volatilization,
- Erosion,
- Deposition (contaminant sinks),
- Weathering of parent material with subsequent transport, and/or
- Water transport:
    - in solution,
    - as suspended material in the water, and
    - bulk transport of solid material.

Several biological processes also affect contaminant fate and transport in the environment:

- Bioaccumulation,
- Biodegradation,
- Biological transformation,[4]
- Food chain transfers, and/or
- Excretion.

[3] The product might be more or less toxic than the parent compound.

[4] The product might be more or less toxic than the parent compound.

Additional information should be gathered on past as well as current mechanisms of contaminant release from source areas at the site. The mechanisms of release along with the chemical and physical form of a contaminant can affect its fate, transport, and potential for reaching ecological receptors.

A contaminant flow diagram (or exposure pathway diagram) comprises a large part of the conceptual model, as illustrated in Section 3.6. A contaminant flow diagram originates at the primary contaminant source(s) and identifies primary release mechanisms and contaminant transport pathways. The release and movement of the contaminants can create secondary sources (e.g., contaminated sediments in a river; see Example 3-1), and even tertiary sources.

The above information is used to evaluate where the contaminants are likely to partition in the environment, and the bioavailability of the contaminant (historically, currently, or in the future). As indicated in Section 3.2, it might be possible for the risk assessment team and the risk manager to use this information to replace some of the conservative assumptions used in the screening-level risk assessment and to eliminate additional chemicals from further evaluation at this point. Any such negotiations must be documented in the baseline risk assessment.

3.4.2 Ecosystems Potentially at Risk

The ecosystems or habitats potentially at risk depend on the ecological setting of a site. An initial source of information on the ecological setting of a site is the data collected during the preliminary site visit and characterization (Step 1), including the site ecological checklist (Appendix B). The site description should provide answers to several questions including:

- What habitats (e.g., maple-beech hardwood forest, early-successional fields) are present?
- What types of water bodies are present, if any?
- Do any other habitats listed in Exhibit 1-1 exist on or adjacent to the site?

While adequately documented information should be used, it is not critical that complete site setting information be collected during this phase of the risk assessment. However, it is important that habitats at the site are not overlooked; hence, a site visit might be needed to supplement the one conducted during the screening risk assessment. If a habitat actually present on the site is omitted during the problem formulation phase, this step might need to be repeated later when the habitat is found, resulting in delays and additional costs for the risk assessment.

EXAMPLE 3-1
Exposure Pathway Model DDT Site

An abandoned pesticide production facility had released DDT to soils through poor handling practices during its operation. Due to erosion of contaminated soils, DDT migrated to stream sediments. The contaminated sediments represent a secondary source that might affect benthic organisms through direct contact or ingestion. Benthic organisms that have accumulated DDT can be consumed by fish, and fish that have accumulated DDT can be consumed by piscivorous birds, which are considered a valuable component of the local ecosystem. This example illustrates how contaminant transport is traced from a primary source to a secondary source and from there through a food chain to an exposure point that can affect an assessment endpoint.

Available information on ecological effects of contaminants (see Section 3.3) can help focus the assessment on specific ecological resources that should be evaluated more thoroughly, because some groups of organisms can be more sensitive than others to a particular contaminant. For example, a species or group of species could be physiologically sensitive to a particular contaminant (e.g., the contaminant might interfere with its vascular system); or, the species might not be able to metabolize and detoxify the particular contaminant(s) (e.g., honey bees and grass shrimp cannot effectively biodegrade PAHs, whereas fish generally can). Alternatively, an already-stressed population (e.g., due to habitat degradation) could be particularly sensitive to any added stresses.

Variation in sensitivity should not be confused with variation in exposure, which can result from behavioral and dietary differences among species. For example, predators can be exposed to higher levels of contaminants that biomagnify in food chains than herbivores. A specialist predator could feed primarily on one prey type that is a primary receptor of the contaminant. Some species might preferentially feed in a habitat where the contaminant tends to accumulate. On the other hand, a species might change its behavior to avoid contaminated areas. Both sensitivity to toxic effects of a contaminant and behaviors that affect exposure levels can influence risks for particular groups of organisms.

3.4.3 Complete Exposure Pathways

The potentially complete exposure pathways identified in Steps 1 and 2 are described in more detail in Step 3 on the basis of the refined contaminant fate and transport evaluations (Section 3.4.1) and evaluation of potential ecological receptors (Section 3.4.2).

Some of the potentially complete exposure pathways identified in Steps 1 and 2 might be ruled out from further consideration at this time. Sometimes, additional exposure pathways might be identified, particularly those originating from secondary sources. Any data gaps that result in questions about whether an exposure pathway is complete should be identified, and the type of data needed to answer those questions should be described to assist in developing the WP and SAP in Step 4.

During Step 3, the potential for food-chain exposures deserves particular attention. Some contaminants are effectively transferred through food chains, while others are not. To illustrate this point, copper and DDT are compared in Example 3-2.

3.5 SELECTION OF ASSESSMENT ENDPOINTS

As noted in the introduction to this guidance, an assessment endpoint is "an explicit expression of the environmental value that is to be protected" (U.S. EPA, 1992a). In human health risk assessment, only one species is evaluated, and cancer and noncancer effects are the usual assessment endpoints. Ecological risk assessment, on the other hand, involves multiple species that are likely to be exposed to differing degrees and to respond differently to the same contaminant. Nonetheless, it is not practical or possible to directly evaluate risks to all of the individual components of the ecosystem at a site. Instead, assessment endpoints focus the risk assessment on particular components of the ecosystem that could be adversely affected by contaminants from the site.

EXAMPLE 3-2
Potential for Food Chain Transfer Copper and DDT Sites

Copper can be toxic in aquatic ecosystems and to terrestrial plants. However, it is an essential nutrient for both plants and animals, and organisms can regulate internal copper concentrations within limits. For this reason, copper tends not to accumulate in most organisms or to biomagnify in food chains, and thus tends not to reach levels high enough to cause adverse responses through food chain transfer to upper-trophic-level organisms. (Copper is known to accumulate by several orders of magnitude in phytoplankton and in filter-feeding mollusks, however, and thus can pose a threat to organisms that feed on those components of aquatic ecosystems; U.S. EPA, 1985a.) In contrast, DDT, a contaminant that accumulates in fatty tissues, can biomagnify in many different types of food chains. Upper-trophic-level species (such as predatory birds), therefore, are likely to be exposed to higher levels of DDT through their prey than are lower-trophic-level species in the ecosystem.

The selection of assessment endpoints includes discussion between the lead risk assessor and the risk manager concerning management policy goals and ecological values. The lead risk assessor and risk manager should seek input from the regional BTAG, PRPs, and other stakeholders associated with a site when identifying assessment endpoints for a site. Stakeholder input at this stage will help ensure that the risk manager can readily defend the assessment endpoints when making decisions for the site. *ECO Update Volume 3, Number 1*, briefly summarizes the process of selecting assessment endpoints (U.S. EPA, 1995b).

Individual assessment endpoints usually encompass a group of species or populations with some common characteristics, such as a specific exposure route or contaminant sensitivity. Sometimes, individual assessment endpoints are limited to one species (e.g., a species known to be particularly sensitive to a site contaminant). Assessment endpoints can also encompass the typical structure and function of biological communities or ecosystems associated with a site.

Assessment endpoints for the baseline ecological risk assessment must be selected based on the ecosystems, communities, and/or species potentially present at the site. The selection of assessment endpoints depends on:

(1) The contaminants present and their concentrations;
(2) Mechanisms of toxicity of the contaminants to different groups of organisms;
(3) Ecologically relevant receptor groups that are potentially sensitive or highly exposed to the contaminant and attributes of their natural history; and
(4) Potentially complete exposure pathways.

Thus, the process of selecting assessment endpoints can be intertwined with other phases of problem formulation. The risk assessment team must think through the contaminant mechanism(s) of ecotoxicity to determine what receptors will or could be at risk. This understanding must include how the adverse effects of the contaminants might be expressed (e.g., eggshell thinning in birds), as well as how the chemical and physical form of the contaminants influence bioavailability and the type and magnitude of adverse response (e.g., inorganic versus organic mercury).

The risk assessment team also should determine if the contaminants can adversely affect organisms in direct contact with the contaminated media (e.g., direct exposure to water, sediment, soil) or if the contaminants accumulate in food chains, resulting in adverse effects in organisms that are not directly exposed or are minimally exposed to the original contaminated media (indirect exposure). The team should decide if the risk assessment should focus on toxicity resulting from direct or indirect exposures, or if both must be evaluated.

Broad assessment endpoints (e.g., protecting aquatic communities) are generally of less value in problem formulation than specific assessment endpoints (e.g., maintaining aquatic community composition and structure downstream of a site similar to that upstream of the site). Specific assessment endpoints define the ecological value in sufficient detail to identify the measures needed to answer specific questions or to test specific hypotheses. Example 3-3 provides three examples of assessment endpoint selection based on the hypothetical sites in Appendix A.

The formal identification of assessment endpoints is part of the SMDP for this step. Regardless of the level of effort to be expended on the subsequent phases of the risk assessment, the assessment endpoints identified are critical elements in the design of the ecological risk assessment and must be agreed upon as the focus of the risk assessment. Once assessment endpoints have been selected, testable hypotheses and measurement endpoints can be developed to determine whether or not a potential threat to the assessment endpoints exists. Testable hypotheses and measurement endpoints cannot be developed without agreement on the assessment endpoints among the risk manager, risk assessors, and other involved professionals.

EXAMPLE 3-3
Assessment Endpoint Selection DDT, Copper, and PCB Sites

DDT Site

An assessment endpoint such as "protection of the ecosystem from the effects of DDT" would give little direction to the risk assessment. However, "protection of piscivorous birds from eggshell thinning due to DDT exposure" directs the risk assessment toward the food-chain transfer of DDT that results in eggshell thinning in a specific group of birds. This assessment endpoint provides the foundation for identifying appropriate measures of effect and exposure and ultimately the design of the site investigation. It is not necessary that a specific species of bird be identified on site. It is necessary that the exposure pathway exists and that the presence of a piscivorous bird could be expected.

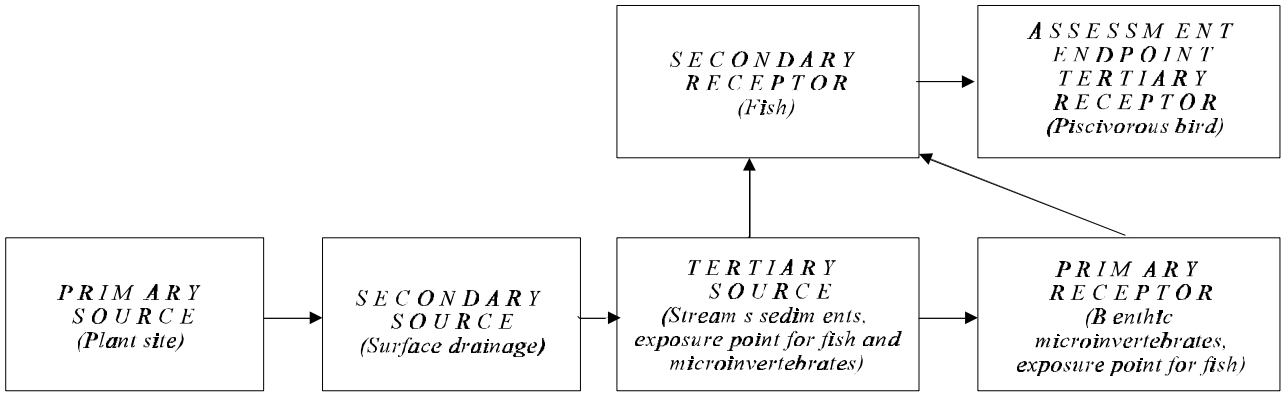
Copper Site

Copper can be acutely or chronically toxic to organisms in an aquatic community through direct exposure of the organisms to copper in the water and sediments. Threats of copper toxicity to higher-trophic-level organisms are unlikely to exceed threats to organisms at the base of the food chain, because copper is an essential nutrient which is effectively regulated by most organisms if the exposure is below immediately toxic levels. Aquatic plants (particularly phytoplankton) and mollusks, however, are poor at regulating copper and might be sensitive receptors or effective in transferring copper to the next trophic level. In addition, fish fry can be very sensitive to copper in water. Based on these receptors and the potential for both acute and chronic toxicity, an appropriate general assessment endpoint for the system could be the maintenance of aquatic community composition. An operational definition of the assessment endpoint for this site would be pond fish and invertebrate community composition similar to that of other ponds of similar size and characteristics in the area.

PCB Site

The primary ecological threat of PCBs in ecosystems is not through direct exposure and acute toxicity. Instead, PCBs bioaccumulate in food chains and can diminish reproductive success in some vertebrate species. PCBs have been implicated as a cause of reduced reproductive success of piscivorous birds (e.g., cormorants, terns) in the Great Lakes (Kubiak et al., 1989; Fox et al., 1991) and of mink along several waterways (Aulerich and Ringer, 1977; Foley et al., 1988). Therefore, reduced reproductive success in high-trophic-level species exposed via their diet is a more appropriate assessment endpoint than either toxicity to organisms via direct exposure to PCBs in water, sediments, or soils, or reproductive impairment in lower-trophic-level species.

EXAMPLE 3-5
Conceptual Model Diagram DDT
Site



3.6 THE CONCEPTUAL MODEL AND RISK QUESTIONS

The site conceptual model establishes the complete exposure pathways that will be evaluated in the ecological risk assessment and the relationship of the measurement endpoints to the assessment endpoints. In the conceptual model, the possible exposure pathways are depicted in an exposure pathway diagram and must be linked directly to the assessment endpoints identified in Section 3.5. Developing the conceptual model and risk questions are described in Sections 3.6.1 and 3.6.2, respectively. Selection of measurement endpoints, completing the conceptual model, is described in Step 4.

3.6.1 Conceptual Model

Based on the information obtained from Steps 1 and 2, knowledge of the contaminants present, the exposure pathway diagram, and the assessment endpoints, an integrated conceptual model is developed (see Example 3-4). The conceptual model includes a contaminant fate-and-transport diagram that traces the contaminants' movement from sources through the ecosystem to receptors that include the assessment endpoints (see Example 3-5). Contaminant exposure pathways that do not lead to a species or group of species associated with the proposed assessment endpoint indicate that either:

(1) There is an incomplete exposure pathway to the receptor(s) associated with the proposed assessment endpoint; or

(2) There are missing components or data necessary to demonstrate a complete exposure pathway.

If case (1) is true, the proposed assessment endpoint should be reevaluated to determine if it is an appropriate endpoint for the site. If case (2) is true, then additional field data could be needed to evaluate contaminant fate and transport at the site. Failure to identify a complete exposure pathway that does exist at the site can result in incorrect conclusions or in extra time and effort being expended on a supplementary investigation.

As indicated in Section 3.5, appropriate assessment endpoints differ from site to site, and can be at one or more levels of biological organization. At any particular site, the appropriate assessment endpoints might involve local populations of a particular species, community-level integrity, and/or habitat preservation. The site conceptual model must encompass the level of biological organization appropriate for the assessment endpoints for the site. The conceptual model can use assumptions that generally represent a group of organisms or ecosystem components.

The intent of the conceptual model is not to describe a particular species or site exactly as much as it is to be systematic, representative, and conservative where information is lacking (with assumptions biased to be more likely to overestimate than to underestimate risk). For example, it is not necessary or even recommended to develop new test protocols to use species that exist a site to test the toxicity of site media (See Step 4). Species used in standardized laboratory toxicity tests (e.g., fathead minnows, *Hyallela* amphipods) usually are adequate surrogates for species in their general taxa and habitat at the site.

EXAMPLE 3-4
Description of the Conceptual Model DDT Site

One of the assessment endpoints selected for the DDT site (Appendix A) is the protection of piscivorous birds. The site conceptual model includes the release of DDT from the spill areas to the adjacent stream, followed by food chain accumulation of DDT from the sediments and water through the lower trophic levels to forage fish in the stream. The forage fish are the exposure point for piscivorous birds. Eggshell thinning was selected as the measure of effect. During the literature review of the ecological effects of DDT, toxicity studies were found that reported reduced reproductive success (i.e., number of young fledged) in birds that experienced eggshell thinning of 20 percent or more (Anderson and Hickey, 1972; Dilworth et al., 1972). Based on those data, the lead risk assessor and risk manager agreed that eggshell thinning of 20 percent or more would be considered an adverse effect for piscivorous birds.

Chronic DDT exposure can also reduce some animals' ability to escape predation. Thus, DDT can indirectly increase the mortality rate of these organisms by making them more susceptible to predators (Cooke, 1971; Krebs et al., 1974). That effect of DDT on prey also can have an indirect consequence for the predators. If predators are more likely to capture the more contaminated prey, the predators could be exposed to DDT at levels higher than represented in the average prey population.

3.6.2 Risk Questions

Ecological risk questions for the baseline risk assessment at Superfund sites are basically questions about the relationships among assessment endpoints and their predicted responses when exposed tocontaminants. The risk questions should be based on the assessment endpoints and provide a basis for developing the study design (Step 4) and for evaluating the results of the site investigation in the analysis phase (Step 6) and during risk characterization (Step 7).

The most basic question applicable to virtually all Superfund sites is whether site-related contaminants are causing or have the potential to cause adverse effects on the assessment endpoint(s). To use the baseline ecological risk assessment in the FS to evaluate remedial alternatives, it is helpful if the specific contaminant(s) responsible can be identified. Thus refined, the question becomes "does (or could) chemical X cause adverse effects on the assessment endpoint?" In general, there are four lines of evidence that can be used to answer this question:

(1) Comparing estimated or measured exposure levels to chemical X with levels that are known from the literature to be toxic to receptors associated with the assessment endpoints;
(2) Comparing laboratory bioassays with media from the site and bioassays with media from a reference site;
(3) Comparing *in situ* toxicity tests at the site with *in situ* toxicity tests in a reference body of water; and
(4) Comparing observed effects in the receptors associated with the site with similar receptors at a reference site.

These lines of evidence are considered further in Step 4, as measurement endpoints are selected to complete the conceptual model and the site-specific study is designed.

3.7 SCIENTIFIC/MANAGEMENT DECISION POINT (SMDP)

At the conclusion of Step 3, there is a SMDP. The SMDP consists of agreement on four items: contaminants of concern, assessment endpoints, exposure pathways, and risk questions. Those items can be summarized with the assistance of the diagram of the conceptual model. Without agreement between the risk manager, risk assessors, and other involved professionals on the conceptual model to this point, measurement endpoints cannot be selected, and a site study cannot be developed effectively. Example 3-5 shows the conceptual model for the DDT site example in Appendix A.

HIGHLIGHT 3-3
Definitions:
Null and Test Hypotheses

Null hypothesis: Usually a hypothesis of no differences between two populations formulated for the express purpose of being rejected.

Test (or alternative) hypothesis: An operational statement of the investigator's research hypothesis.

When appropriate, formal hypothesis testing is preferred to make explicit what error rates are acceptable and what magnitude of effect is considered biologically important. However, it might not be practical for many assessment endpoints or be the only acceptable way to state questions about those endpoints. See Example 4-1 in the next chapter.

3.8 SUMMARY

By combining information on: (1) the potential contaminants present; (2) the ecotoxicity of the contaminants; (3) environmental fate and transport; (4) the ecological setting; and (5) complete exposure pathways, an evaluation is made of what aspects of the ecosystem at the site could be at risk and what the adverse ecological response could be. "Critical exposure pathways" are based on: (1) exposure pathways to sensitive species' populations or communities; and (2) exposure levels associated with predominant fate and transport mechanisms at a site.

Based on that information, the risk assessors and risk manager agree on assessment endpoints and specific questions or testable hypotheses that, together with the rest of the conceptual model, form the basis for the site investigation. At this stage, site-specific information on exposure pathways and/or the presence of specific species is likely to be incomplete. By using the conceptual model developed thus far, measurement endpoints can be selected, and a plan for filling information gaps can be developed and written into the ecological WP and SAP as described in Step 4.

STEP 4: STUDY DESIGN AND DATA QUALITY OBJECTIVE PROCESS

OVERVIEW

The site conceptual model begun in Step 3, which includes assessment endpoints, exposure pathways, and risk questions or hypotheses, is completed in Step 4 with the development of measurement endpoints. The conceptual model then is used to develop the study design and data quality objectives. The products of Step 4 are the ecological risk assessment WP and SAP, which describe the details of the site investigation as well as the data analysis methods and data quality objectives (DQOs). As part of the DQO process, the SAP specifies acceptable levels of decision errors that will be used as the basis for establishing the quantity and quality of data needed to support ecological risk management decisions.

The lead risk assessor and the risk manager should agree that the WP and SAP describe a study that will provide the risk manager with the information needed to fulfill the requirements of the baseline risk assessment and to incorporate ecological considerations into the site remedial process. Once this step is completed, most of the professional judgment needed for the ecological risk assessment will have been incorporated into the design and details of the WP and SAP. This does not limit the need for qualified professionals in the implementation of the investigation, data acquisition, or data interpretation. However, there should be no fundamental changes in goals or approach to the ecological risk assessment once the WP and SAP are finalized.

Step 4 of the ecological risk assessment establishes the measurement endpoints (Section 4.1), completing the conceptual model begun in Step 3. Step 4 also establishes the study design (Section 4.2) and data quality objectives based on statistical considerations (Section 4.3) for the site assessment that will accompany site-specific studies for the remedial investigation. The site conceptual model is used to identify which points or assumptions in the risk assessment include the greatest degree of conservatism or uncertainty. The field sampling then can be designed to address the risk model parameters that have important effects on the risk estimates (e.g., bioavailability and toxicity of contaminants in the field, contaminant concentrations at exposure points).

The products of Step 4 are the WP and SAP for the ecological component of the field investigations (Section 4.4). Involvement of the BTAG in the preparation, review, and approval of WPs and SAPs can help ensure that the ecological risk assessment is well focused, performed efficiently, and technically correct. The WP and SAP should specify the site conceptual model developed in Step 3, and the measurement endpoints developed in the beginning of Step 4. The WP describes:

- Assessment endpoints;

- Exposure pathways;
- Questions and testable hypotheses;
- Measurement endpoints and their relation to assessment endpoints; and
- Uncertainties and assumptions.

The SAP should describe:

- Data needs;
- Scientifically valid and sufficient study design and data analysis procedures;
- Study methodology and protocols, including sampling techniques;
- Data reduction and interpretation techniques, including statistical analyses; and
- Quality assurance procedures and quality control techniques.

The SAP must include the data reduction and interpretation techniques, because it is necessary to known how the data will be interpreted to specify the number of samples needed. Prior to formal agreement on the WP and SAP, the proposed field sampling plan is verified in Step 5.

4.1 ESTABLISHING MEASUREMENT ENDPOINTS

As indicated in the Introduction, a measurement endpoint is defined as "a measurable ecological characteristic that is related to the valued characteristic chosen as the assessment endpoint" and is a measure of biological effects (e.g., mortality, reproduction, growth) (U.S. EPA, 1992a; although this definition may change—see U.S. EPA 1996a). Measurement endpoints are frequently numerical expressions of observations (e.g., toxicity test results, community diversity measures) that can be compared statistically to a control or reference site to detect adverse responses to a site contaminant. As used in this guidance, measurement endpoints can include measures of exposure (e.g., contaminant concentrations in water) as well as measures of effect. The relationship between measurement and assessment endpoints must be clearly described within the conceptual model and must be based on scientific evidence. This is critical because the assessment and measurement endpoints usually are different endpoints (see the Introduction and Highlight 4-1)

Typically, the number of measurement endpoints that are potentially appropriate for any given assessment endpoint and circumstance is limited. The most appropriate measurement endpoints for an assessment endpoint depend on several considerations, a primary one being how many and which lines of evidence are needed to

HIGHLIGHT 4-1
Importance of Distinguishing Measurement from Assessment Endpoints

If a measurement endpoint is mistaken for an assessment endpoint, the misperception can arise that Superfund is basing a remediation on an arbitrary or esoteric justification. For example, protection of a few invertebrate and algal species could be mistaken as the basis for a remedial decision, when the actual basis for the decision is the protection of the aquatic community as a whole (including higher-trophic-level game fish that depend on lower trophic levels in the community), as indicated by a few sensitive invertebrate and algal species.

support risk-management decisions at the site (see Section 3.6.2). Given the potential ramifications of site actions, the site risk manager might want to use more than one line of evidence to identify site-specific thresholds for effects. The risk manager and risk assessors must consider the utility of each type of data given the cost of collecting those data and the likely sensitivity of the risk estimates to the data.

There are some situations in which it might only be necessary or possible to compare estimated or measured contaminant exposure levels at a site to ecotoxicity values derived from the literature. For example, for contaminants in surface waters for which there are state water-quality standards, exceedance of the standards indicates that remediation to reduce contaminant concentrations in surface waters to below these levels could be needed whether impacts are occurring or not. For assessment endpoints for which impacts are difficult to demonstrate in the field (e.g., because of high natural variability), and toxicity tests are not possible (e.g., food-chain accumulation is involved), comparing environmental concentrations with a well-supported ecotoxicity value might have to suffice.

A bioassay using contaminated media from the site can suffice if the risk manager and risk assessor agree that laboratory tests with surrogate species will be taken as indicative of likely effects on the assessment endpoint. For sites with complex mixtures of contaminants without robust ecotoxicity values and high natural variability in potential measures for the assessment endpoint, either laboratory or *in situ* toxicity testing might be the best technique for evaluating risks to the assessment endpoint. For inorganic substances in soils or sediments, bioassays often are needed to determine the degree to which a contaminant is bioavailable at a particular site. Laboratory toxicity tests can indicate the potential for adverse impacts in the field, while *in situ* toxicity testing with resident organisms can provide evidence of actual impacts occurring in the field.

Sometimes more than one line of evidence is needed to reasonably demonstrate that contaminants from a site are likely to cause adverse effects on the assessment endpoint. For example, total recoverable copper in a surface water body to which a water quality standard did not apply could exceed aquatic ecotoxicity values, but not cause adverse effects because the copper is only partially bioavailable or because the ecotoxicity value is too conservative for the particular ecosystem. Additional evidence from bioassays or community surveys could help resolve whether the copper is actually causing adverse effects (See Example 4-1). Alternatively, if stream community surveys indicate impairment of community structure downstream of a site, comparing contaminant concentrations with aquatic toxicity values can help identify which contaminants are most likely to be causing the effect. When some lines of evidence conflict with others, professional judgment is needed to determine which data should be considered more reliable or relevant to the questions.

Once there is agreement on which lines of evidence are required to answer questions concerning the assessment endpoint, the measurement endpoints by which the questions or test hypotheses will be examined can be selected.

Each measurement endpoint should represent the same exposure pathway and toxic mechanism of action as the assessment endpoint it represents; otherwise, irrelevant exposure pathways or toxic mechanisms might be evaluated. For example, if a contaminant primarily causes damage to vertebrate kidneys, the use of daphnids (which do not have kidneys) would be inappropriate.

EXAMPLE 4-1
Lines of Evidence Copper Site

Primary question: Are ambient copper levels in sediments causing adverse effects in benthic organisms in the pond?

Possible lines of evidence phrased as test hypotheses:

(1) Mortality in early life stages of benthic aquatic insects in contact with sediments from the site significantly exceeds mortality in the same kinds of organisms in contact with sediments from a reference site (e.g., $p \leq 0.1$).

(2) Mortality in *in situ* toxicity tests in sediments at the pond significantly exceeds mortality in *in situ* toxicity tests in sediments at a reference pond (e.g., $p \leq 0.1$).

(3) There are significantly fewer numbers of benthic aquatic insect species present per m^2 of sediment at the pond near the seep than at the opposite side of the pond (e.g., $p \leq 0.1$).

Statistical and biological significance: Differences in the incidence of adverse effects between groups of organisms exposed to contaminants from the site and groups not exposed might be statistically significant, but not biologically important, depending on the endpoint and the power of the statistical test. Natural systems can sustain some level of perturbation without changing in structure or function. The risk assessor needs to evaluate what level of effect will be considered biologically important. Given the limited power of small sample sizes to detect an effect, the risk assessor might decide that any difference that is statistically detectable at a p level of 0.1 or less is important biologically.

Potential measurement endpoints in toxicity tests or in field studies should be evaluated according to how well they can answer questions about the assessment endpoint or support or refute the hypotheses developed for the conceptual model. Statistical considerations, including sample size and statistical power described in Section 4.3, also must be considered in selecting the measurement endpoints. The following subsections describe additional considerations for selecting measurement endpoints, including species/community/habitat (Section 4.1.1), relationship to the contaminant(s) of concern (Section 4.1.2), and mechanisms of ecotoxicity (Section 4.1.3).

4.1.1 Species/Community/Habitat Considerations

The function of a measurement endpoint is to represent an assessment endpoint for the site. The measurement endpoint must allow clear inferences about potential changes in the assessment endpoint. Whenever assessment and measurement endpoints are not the same (which usually is the case), measurement endpoints should be selected to be inclusive of risks to all of the species, populations, or groups included in the assessment endpoint that are not directly measured. In other words, the measurement endpoint should be representative of the assessment endpoint for the site and not lead to an underestimate of risk to the assessment endpoint. Example 4-2 illustrates this point for the DDT site in Appendix A.

In selecting a measurement endpoint, the species and life stage, population, or community chosen should be the one(s) most susceptible to the contaminant for the assessment endpoint in question. For species and populations, this selection is based on a review of the species: (1) life history; (2) habitat utilization; (3) behavioral characteristics; and (4) physiological parameters. Selection of measurement endpoints also should be based on which routes of exposure are likely. For communities, careful evaluation of the contaminant fate and transport in the environment is essential.

4.1.2 Relationship of the Measurement Endpoints to the Contaminant of Concern

Additional criteria to consider when selecting measurement endpoints are inherent properties (such as the physiology or behavioral characteristics of the species) or life history parameters that make a species useful in evaluating the effects of site-specific contaminants.

HIGHLIGHT 4-2
Terminology and Definitions

In the field of ecotoxicology, there historically have been multiple definitions for some terms, including definitions for direct effects, indirect effects, acute effects, chronic effects, acute tests, and chronic tests. This multiplicity of definitions has resulted in misunderstandings and inaccurate communication of study designs. Definitions of these and other terms, as they are used in this document, are provided in the glossary. When consulting other reference materials, the user should evaluate how the authors defined terms.

For example, *Chironomus tentans* (a species of midge that is used as a standard sediment toxicity testing species in the larval stage) is considered more tolerant of metals contamination than is *C. riparius*, a similar species (Klemm et al., 1990; Nebeker et al., 1984; Pascoe et al., 1989). To assess the effects of exposure of benthic communities to metal-contaminated sediment, *C. riparius* might be the better species to use as a test organism for many aquatic systems to ensure that risks are not underestimated. In general, the most sensitive of the measurement endpoints appropriate for inferring risks to the assessment endpoint should be used. If all else is equal, however, species that are commonly used in the laboratory are preferred over non-standard laboratory species to improve test precision.

Some species have been identified as being particularly sensitive to certain contaminants. For example, numerous studies have demonstrated that mink are among the most sensitive of the tested mammalian species to the toxic effects of PCBs (U.S. EPA, 1995a). Species that rely on quick reactions or behavioral responses to avoid predators can be particularly sensitive to contaminants affecting the central nervous system, such as mercury. Thus, the sensitivity of the measurement endpoint relative to the assessment endpoint should be considered for each contaminant of concern.

4.1.3 Mechanisms of Ecoxicity

A contaminant can exert adverse ecological effects in many ways. First, a contaminant might affect an organism after exposure for a short period of time (acute) or after exposure over an extended period of time (chronic). Second, the effect of a contaminant could be lethal (killing the organism) or sublethal (causing adverse effects other than death, such as reduced growth, behavioral changes, etc.). Sublethal effects can reduce an organism's lifespan or reproductive success. For example, if a contaminant reduces the reaction speed of a prey species, the prey can become more susceptible to

predation. Third, a contaminant might act directly or indirectly on an organism. Direct effects include lethal or sublethal effects of the chemical on the organism. Indirect effects occur when the contaminant damages the food, habitat, predator-prey relationships, or competition of the organism in its community.

Mechanisms of ecotoxicity and exposure pathways have already been considered during problem formulation and identification of the assessment endpoints. However, toxicity issues are revisited when selecting appropriate measurement endpoints to ensure that they measure the assessment endpoint's toxic response of concern.

4.2 STUDY DESIGN

In Section 4.1, one or more lines of evidence that could be used to answer questions or to test hypotheses concerning the assessment endpoint(s) were identified. This section provides recommendations on how to design a field study for: bioaccumulation and field tissue residue studies (Section 4.2.1); population/community evaluations (Section 4.2.2); and toxicity testing (Section 4.2.3). A thorough understanding of the strengths and limitations of these types of field studies is necessary to properly design any investigation.

Typically, no one line of evidence can stand on its own. Analytic chemistry on co-located samples and other lines of evidence are needed to support a conclusion. When population/community evaluations are coupled with toxicity testing and media chemistry, the procedure often is referred to as a triad approach (Chapman et al., 1992; Long and Chapman, 1985). This method has proven effective in defining the area affected by contaminants in sediments of several large bays and estuaries.

The development of exposure-response relationships is critical for evaluating risk management options; thus, for all three types of studies, sampling is applied to a contamination gradient when possible as well as compared to reference data. Reference data are baseline values or characteristics that should represent the site in the absence of contaminants released from the site. Reference data might be data collected from the site before contamination occurred or new data collected from a reference site.

The reference site can be the least impacted (or unimpacted) area of the Superfund site or a nearby site that is ecologically similar, but not affected by the site's contaminants. For additional information on selecting and using reference information in Superfund ecological risk assessments, see *ECO Update Volume 2, Number 1* (U.S. EPA, 1994e).

The following subsections present a starting point for selecting an appropriate study design for the different types of biological sampling that might apply to the site investigation.

EXAMPLE 4-2
Selecting Measurement Endpoints DDT Site

As described in Example 3-1, one of the assessment endpoints selected for the DDT site is the protection of piscivorous birds from egg-shell thinning due to DDT exposure. The belted kingfisher was selected as a piscivorous bird with the smallest home range that could utilize the area of the site, thereby maximizing the calculated dose to a receptor. In this illustration, the kingfishers are used as the most highly exposed of the piscivorous birds potentially present. Thus, one can conclude that, if the risk assessment shows no threat of eggshell thinning to the kingfisher, there should be minimal or no threat to other piscivorous birds that might utilize the site. Thus, eggshell thinning in belted kingfishers is an appropriate measurement endpoint for this site.

4.2.1 Bioaccumulation and Field Tissue Residue Studies

Bioaccumulation and field tissue residue studies typically are conducted at sites where contaminants are likely to accumulate in food chains. The studies help to evaluate contaminant exposure levels associated with measures of effect for assessment endpoint species.

The degree to which a contaminant is transferred through a food chain can be evaluated in several ways. The most common type of study reported in the literature is a contaminant bioaccumulation (uptake) study. As indicated in Section 2.2.1, the most conservative BAF values identified in the literature generally are used to estimate bioaccumulation in Step 2 of the screening-level risk assessment. Where the potential for overestimating bioaccumulation by using conservative literature values to represent the site is substantial, additional evaluation of the literature for values more likely to apply to the site or a site-specific tissue residue study might be advisable.

A tissue residue study generally is conducted on organisms that are in the exposure pathway (i.e., food chain) associated with the assessment endpoint. Data seldom are available to link tissue residue levels in the sampled organisms to adverse effects in those organisms. Literature toxicity studies usually associate effects with an administered dose (or data that can be converted to an administered dose), not a tissue residue level. Thus, the purpose of a field tissue residue study usually is to measure contaminant concentrations in foods consumed by the species associated with the assessment endpoint. This measurement minimizes the uncertainty associated with estimating a dose (or intake) to thatspecies,particularly in situations in which several media and trophic levels are in the exposure pathway.

The concentration of a contaminant in the primary prey/food also should be linked to an exposure concentration from a contaminated medium (e.g., soil, sediment, water), because it is the medium, not the food chain, that will be remediated. Thus, contaminant concentrations must be measured in environmental media at the same locations at which the organisms are collected along contaminant gradients and at reference locations. Co-located samples of the contaminated medium and organisms are needed to establish a correlation between the tissue residue levels and contamination levels in the

medium under evaluation; these studies are most effective if conducted over a gradient of contaminant concentrations. In addition, tissue residues from sessile organisms (e.g., rooted plants, clams) are easier to attribute to specific contaminated areas than are tissue residues from mobile organisms (e.g., large fish). Example 4-3 illustrates these concepts using the DDT site example in Appendix A

EXAMPLE 4-3
Tissue Residue Studies DDT Site

In the DDT site example, a forage fish (e.g., creek chub) will be collected at several locations with known DDT concentrations in sediments. The forage fish will be analyzed for body burdens of DDT, and the relationship between the DDT levels in the sediments and the levels in the forage fish will be established. The forage fish DDT concentrations can be used to evaluate the DDT threat to piscivorous birds feeding on the forage fish at each location. Using the DDT concentrations measured in fish that correspond to a LOAEL and NOAEL for adverse effects in birds and the relationship between the DDT levels in the sediments and in the forage fish, the corresponding sediment contamination levels can be estimated. Those sediment DDT concentrations can then be used to estimate a cleanup level that would reduce threats of eggshell thinning to piscivorous birds.

Although it might seem obvious, it is important to confirm that the organisms examined for tissue residue levels are in the exposure pathways of concern established by the conceptual model. Food items targeted for collection should be those that are likely to constitute a large portion of the diet of the species of concern (e.g., new growth on maple trees, rather than cattails, as a food source for deer) and/or represent pathways of maximum exposure. If not, erroneous conclusions or study delays and added costs can result. Because specific organisms often can only be captured in one season, the timing of the study can be critical, and failure to plan accordingly can result in serious site management difficulties.

There are numerous factors that must be considered when selecting a species in which to measure contaminant residue levels. Several investigators have discussed the "ideal" characteristics of the species to be collected and analyzed. The recommendations of Phillips (1977, 1978) include that the species selected should be:

(1) Able to accumulate the chemical of concern without being adversely affected by the levels encountered at the site;
(2) Sedentary (small home range) in order to be representative of the area of collection;
(3) Abundant in the study area; and
(4) Of reasonable size to give adequate tissue for analysis (e.g., 10 grams for organic analysis and 0.5 gram for metal analysis for many laboratories (Roy F. Weston, Inc.,1994).

Additional considerations for some situations would be that the species is:

(5) Sufficiently long-lived to allow for sampling more than one age class; and

(6) Easy to sample and hardy enough to survive in the laboratory (allowing for the organisms to eliminate contaminants from their gastrointestinal tract prior to analysis, if desired, and allowing for laboratory studies on the uptake of the contaminant).

It is usually not possible or necessary to find an organism that fulfills all of the above requirements. The selection of an organism for tissue analysis should balance these characteristics with the hypotheses being tested, knowledge of the contaminants' fate and transport, and the practicality of using the particular species. In the following sections, several of the factors mentioned above are described in greater detail.

Ability to accumulate the contaminant. The objectives of a tissue residue study are (1) to measure bioavailability directly; (2) to provide site-specific estimates of exposure to higher-trophic-level organisms; and (3) to relate tissue residue levels to concentrations in environmental media (e.g., in soil, sediment, or water). Sometimes these studies also can be used to link tissue residue levels with observed effects in the organisms sampled. However, in a "pure" accumulation study, the species selected for collection and tissue analysis should be ones that can accumulate a contaminant(s) without being adversely affected by the levels encountered in the environment. While it is difficult to evaluate whether or not a population in the field is affected by accumulation of a contaminant, it is important to try. Exposure that results in adverse responses might alter the animal's feeding rates or efficiency, diet, degree of activity, or metabolic rate, and thereby influence the animal's daily intake or accumulation of the contaminant and the estimated BAF. For example, if the rate of bioaccumulation of a contaminant in an organism decreases with increasing environmental concentrations (e.g., its toxic effects reduce food consumption rates), using a BAF determined at low environmental concentrations to estimate bioaccumulation at high environmental concentrations would overestimate risk. Conversely, if bioaccumulation increased with increasing environmental concentrations (e.g., its toxic effects impair the organisms' ability to excrete the contaminant), using a BAF determined at low environmental concentrations would underestimate risks at higher environmental concentrations.

Consideration of the physiology and biochemistry of the species selected for residue analysis also is important. Some species can metabolize certain organic contaminant(s) (e.g., fish can metabolize PAHs). If several different types of prey are consumed by a species of concern, it would be more appropriate to analyze prey species that do not metabolize the contaminant.

Home range. When selecting species for residue analyses, one should be confident that the contaminant levels found in the organism depend on the contaminant levels in the environmental media under evaluation. Otherwise, valid conclusions cannot be drawn about ecological risks posed by contaminants at the site. The home range, particularly the foraging areas within the home range, and movement patterns of a species are important in making this determination. Organisms do not utilize the environment uniformly. For species that have large home ranges or are migratory, it can be difficult to evaluate potential exposure to contaminants at the site. Attribution of contaminant levels in an organism to contaminant levels in the surrounding environment is easiest for animals with small home and

foraging ranges and limited movement patterns. Examples of organisms with small home ranges include young-of-the-year fish, burrowing crustacea (such as fiddler crabs or some crayfish), and small mammals.

Species also should be selected for residue analysis to maximize the overlap between the area of contamination and the species' home range or feeding range. This provides a conservative evaluation of potential exposure levels. The possibility that a species' preferred foraging areas within a home range overlap the areas of maximum contamination also should be considered.

Population size. A species selected for tissue residue analysis should be sufficiently abundant at the site that adequate numbers (and sizes) of individuals can be collected to support the tissue mass requirements for chemical analysis and to achieve the sample size needed for statistical comparisons. The organisms actually collected should be not only of the same species, but also of similar age or size to reduce data variability when BAFs are being evaluated. The practicality of using a particular species is evaluated in Step 5.

Size/composites. When selecting species in which to measure tissue residue levels, it is best to have individual animals large enough for chemical analysis, without having to pool (combine) individuals prior to chemical analysis. However, composite samples will be needed if individuals from the species selected cannot yield sufficient tissue for the required analytical methods. Linking contaminant levels in organisms to concentrations in environmental media is easier if composites are made up of members of the same species, sex, size, and age, and therefore exhibit similar accumulation characteristics. When deciding whether or not to pool samples, it is important to consider what impact the loss of information on variability of contaminant levels along these dimensions will have on data interpretation. The size, age, and sex of the species collected should be representative of the range of prey consumed by the species of concern.

Summary. Although it can be difficult to meet all of the suggested criteria for selecting a species for tissue residue studies, an attempt should be made to meet as many criteria as possible. No formula is available for ranking the factors in order of importance within a particular site investigation because the ranking depends on the study objectives. However, a key criterion is that the organism be sedentary or have a limited home range. It is difficult to connect site contamination to organisms that migrate over great distances or that have extremely large home ranges. Further information on factors that can influence bioaccumulation is available from the literature (e.g., Phillips, 1977, 1978; U.S. EPA, 1995d).

4.2.2 Population/Community Evaluations

Population/community evaluations, or biological field surveys, are potentially useful for both contaminants that are toxic to organisms through direct exposure to the contaminated medium and contaminants that bioaccumulate in food chains. In either case, careful consideration must be given to the mechanism of contaminant effects. Since population/community evaluations are "impact" evaluations, they typically are not predictive. The release of the contaminant must already have occurred and exerted an effect in order for the population/community evaluation to be. an effective tool for a risk assessment.

Population and community surveys evaluate the current status of an ecosystem, often using several measures of population or community structure (e.g., standing biomass, species richness) or function (e.g., feeding group analysis). The most commonly used measures include number of species and abundance of organisms in an ecosystem, although some species are difficult to evaluate. It is difficult to detect changes in top predator populations affected by bioaccumulation of substances in their food chain due to the mobility of top predators. Some species, most notably insects, can develop a tolerance to contaminants (particularly pesticides); in these cases, a population/community survey would be ineffective for evaluating existing impacts. While population/community evaluations can be useful, the risk assessors should consider the level of effort required as well as the difficulty in accounting for natural variability.

A variety of population/community evaluations have been used at Superfund sites. Benthic macroinvertebrate surveys are the most commonly conducted population/community evaluations. There are methods manuals (e.g., U.S. EPA 1989c, 1990a) and publications that describe the technical procedures for conducting these studies. In certain instances, fish community evaluations have proven useful at Superfund sites. However, these investigations typically are more labor-intensive and costly than a comparable macroinvertebrate study. In addition, fish generally are not sensitive measures of the effects of sediment contamination, because they usually are more mobile than benthic macroinvertebrates. Terrestrial plant community evaluations have been used to a limited extent at Superfund sites. For those surveys, it is important to include information about historical land use and physical habitat disruption in the uncertainty analysis.

Additional information on designing field studies and on field study methods can be found in *ECO Update Volume 2, Number 3* (U.S. EPA, 1994d).

Although population- and community-level studies can be valuable, several factors can confound the interpretation of the results. For example, many fish and small mammal populations normally cycle in relation to population density, food availability, and other factors. Vole populations have been known to reach thousands of individuals per acre and then to decline to as low as tens of individuals per acre the following years without an identifiable external stressor (Geller, 1979). It is important that the "noise of the system" be evaluated so that the impacts attributed to chemical contamination at the site are not actually the result of different, "natural" factors. Populations located relatively close to each other can be affected independently: one might undergo a crash, while another is peaking. Physical characteristics of a site can isolate populations so that one population level is not a good indicator of another; for example, a paved highway can be as effective a barrier as a river, and populations on either side can fluctuate independently. Failure to evaluate such issues can result in erroneous conclusions. The level of effort required to resolve some of these issues can make population/community evaluations impractical in some circumstances.

4.2.3 Toxicity Testing

The bioavailability and toxicity of site contaminants can be tested directly with toxicity tests. As with other methods, it is critical that the media tested are in exposure pathways relevant to the assessment endpoint. If the site conceptual model involves exposure of benthic invertebrates to contaminated sediments, then a solid-phase toxicity test using contaminated sediments (as opposed to a water-column exposure test) and an infaunal species would be appropriate. As indicated earlier, the

species tested and the responses measured must be compatible with the mechanism of toxicity. Some common site contaminants are not toxic to most organisms at the same environmental concentrations that threaten top predators because the contaminant biomagnifies in food chains (e.g., PCBs); toxicity tests using contaminated media from the site would not be appropriate for evaluating this type of ecological threat.

There are numerous U.S. EPA methods manuals and ASTM guides and procedures for conducting toxicity tests (see references in the Bibliography). While documented methods exist for a wide variety of toxicity tests, particularly laboratory tests, the risk assessor must evaluate what a particular toxicity test measures and, just as importantly, what it does not measure. Questions to consider when selecting an appropriate toxicity test include:

(1) What is the mechanism of toxicity of the contaminant(s)?

(2) What contaminated media are being evaluated (water, soil, sediment)?

(3) What toxicity test species are available to test the media being evaluated?

(4) What life stage of the species should be tested?

(5) What should the duration of the toxicity test be?

(6) Should the test organisms be fed during the test?

(7) What endpoints should be measured?

There are a limited number of toxicity tests that are readily available for testing environmental media. Many of the aquatic toxicity tests were developed for the regulation of aqueous discharges to surface waters. These tests are useful, but one must consider the original purpose of the test.

New toxicity tests are being developed continually and can be of value in designing a Superfund site ecological risk assessment. However, when non-standard tests are used, complete documentation of the specific test procedures is necessary to support use of the data.

In situ toxicity tests involve placing organisms in locations that might be affected by site contaminants and in reference locations. Non-native species should not be used, because of the risk of their release into the environment in which they could adversely affect (e.g., prey on or outcompete) resident species. *In situ* tests might provide more realistic evidence of existing adverse effects than laboratory toxicity tests; however, the investigator has little control over many environmental parameters and the experimental organisms can be lost to adverse weather or other events (e.g., human interference) at the site or reference location.

For additional information on using toxicity tests in ecological risk assessments, see *ECO Update Volume 2, Numbers 1* and *2* (U.S. EPA, 1994b,c).

4.3 DATA QUALITY OBJECTIVES AND STATISTICAL CONSIDERATIONS

The SAP indicates the number and location of samples to be taken, the number of replicates for each sampling location, and the method for determining sampling locations. In specifying those parameters, the investigator needs to consider, among other things, the DQOs and statistical methods that will be used to analyze the data.

4.3.1 Data Quality Objectives

The DQO process represents a series of planning steps that can be employed throughout the development of the WP and SAP to ensure that the type, quantity, and quality of environmental data to be collected during the ecological investigation are adequate to support the intended application. Problem formulation in Steps 3 and 4 is essentially the DQO process. By employing problem formulation and the DQO process, the investigator is able to define data requirements and error levels that are acceptable for the investigation prior to the collection of data. This approach helps ensure that results are appropriate and defensible for decision making. The specific goals of the general DQO process are to:

- Clarify the study objective and define the most appropriate types of data to collect;
- Determine the most appropriate field conditions under which to collect the data; and
- Specify acceptable levels of decision errors that will be used as the basis for establishing the quantity and quality of data needed to support risk management decisions.

As the discussion of Steps 3 and 4 indicates, those goals are subsumed in the problem formulation phase of an ecological risk assessment. Several U.S. EPA publications provide detailed descriptions of the DQO process (U.S. EPA, 1993c,d,f, 1994f). Because many of the steps of the DQO process are already covered during problem formulation, the DQO process should be reviewed by the investigator and applied as needed.

4.3.2 Statistical Considerations

Sampling locations can be selected "randomly" to characterize an area or non-randomly, as along a contaminant concentration gradient. The way in which sampling locations are selected determines which statistical tests, if any, are appropriate for evaluating test hypotheses.

If a toxicity test is to be used to identify contaminant concentrations in the environment associated with a threshold for adverse effects, the statistical power of the test is important. The threshold for effects is assumed to be between the NOAEL and LOAEL of a toxicity test (see Section 7.3.1). For toxicity tests that use a small number of test and control organisms or for which the toxic response is highly variable, the increase in response rate of the test animals compared with controls often must be relatively high (e.g., 30 to 50 percent increase) for the response to be considered a LOAEL (i.e., statistically increased level of an adverse response compared with control levels). If a NOAEL-to-LOAEL range that might represent a 20 to 50 percent increase in adverse effect is unacceptable (e.g., a population is unlikely to sustain itself with an additional 40 percent mortality), then the power of the study design must be increased, usually by increasing sample size, but sometimes by taking full advantage of all available information to improve the power of the design (e.g., stratified sampling, special tests for trends, etc.). A limitation on the use of toxicity values from the literature is that often, the investigator does not discuss the statistical power of the study design, and hence does not indicate the minimum statistically detectable effect level. Appendix D describes additional statistical considerations, including a description of Type I and Type II error, statistical power, statistical models, and power efficiency.

In evaluating the results of statistical analyses, one should remember that a statistically significant difference relative to a control or reference population does not necessarily imply a biologically important or ecologically significant difference (see Example 4-1).

4.4 CONTENTS OF WORK PLAN AND SAMPLING AND ANALYSIS PLAN

The WP and SAP for the ecological investigation should be developed as part of the initial RI sampling event if possible. If not, the WP and SAP can be developed as an additional phase of the site investigation. In either case, the format of the WP and SAP should be similar to that described by U.S. EPA (1988a, 1989b). Accordingly, those documents should be consulted when developing the ecological investigation WP and SAP.

The WP and SAP are typically written as separate documents. In that case, the WP can be submitted for the risk manager's review so that any concerns with the approach can be resolved prior to the development of the SAP. For some smaller sites, it might be more practical to combine the two documents, in which case, the investigators should discuss the overall objectives and approach with the risk manager to ensure that all parties agree.

The WP and SAP are briefly described in Sections 4.4.1 and 4.4.2, respectively. A plan for testing the SAP before the site WP and SAP are signed and the investigation begins is described in Section 4.4.3.

4.4.1 Work Plan

The purpose of the WP is to document the decisions and evaluations made during problem formulation and to identify additional investigative tasks needed to complete the evaluation of risks to ecological resources. As presented in U.S. EPA (1988a), the WP generally includes the following:

- A general overview and background of the site including the site's physical setting, ecology, and previous uses;
- A summary and analysis of previous site investigations and conclusions;
- A site conceptual model, including an identification of the potential exposure pathways selected for analysis, the assessment endpoints and questions or testable hypotheses, and the measurement endpoints selected for analysis;
- The identification of additional site investigations needed to conduct the ecological risk assessment; and
- A description of assumptions used and the major sources of uncertainty in the site conceptual model and existing information.

The general scope of the additional sampling activities also is presented in the WP. A detailed description of the additional sampling activities is presented in the SAP along with an anticipated schedule of the site activities.

4.4.2 Sampling and Analysis Plan

The SAP typically consists of two components: a field sampling plan (FSP) and a quality assurance project plan (QAPP). The FSP provides guidance for all field work by providing a detailed description of the sampling and data-gathering procedures to be used for the project. The QAPP provides a description of the steps required to achieve the objectives dictated by the intended use of the data.

Field sampling plan. The FSP provides a detailed description of the samples needed to meet the objectives and scope of the investigation outlined in the WP. The FSP for the ecological assessment should be detailed enough that a sampling team unfamiliar with the site would be able to gather all the samples and/or required field data based on the guidelines presented in the document. The FSP for the ecological investigation should include a description of the following elements:

- Sampling type and objectives;
- Sampling location, timing, and frequency;
- Sample designation;

- Sampling equipment and procedures; and
- Sample handling and analysis.

A detailed description of those elements for chemical analyses is provided in Appendix B of U.S. EPA (1988a). Similar specifications should be developed for the biological sampling.

Quality assurance project plan. The objective of the QAPP is to provide a description of the policy, organization, functional activities, and quality control protocols necessary for achieving the study objectives. Highlight 4-3 presents the elements typically contained in a QAPP.

U.S. EPA has prepared guidance on the contents of a QAPP (U.S. EPA, 1987a, 1988a, 1989a). Formal quality assurance and quality control (QA/QC) procedures exist for some types of ecological assessments, for example, for laboratory toxicity tests on aquatic species. For standardized laboratory tests, there are formal QA/QC procedures that specify (1) sampling and handling of hazardous wastes; (2) sources and culturing of test organisms; (3) use of reference toxicants, controls, and exposure replicates; (4) instrument calibration; (5) record keeping; and (6) data evaluation. For other types of ecological assessments, however, QA/QC procedures are less well defined (e.g., for biosurveys of vegetation, terrestrial vertebrates). BTAG members can provide input on appropriate QA/QC procedures based on their experience with Superfund sites.

4.4.3 Field Verification of Sampling Plan and Contingency Plans

For biological sampling, uncontrolled variables can influence the availability of species to be sampled, the efficiency of different types of sampling techniques, and the level of effort required to achieve the sample sizes specified in the SAP. As a consequence, the risk assessor should develop a plan to test the sampling design before the WP and SAP are signed and the site investigationbegins. Otherwise, field sampling during the site investigation could fail to meet the DQOs specified in the SAP, and the study could fail to meet its objectives. Step 5 provides a description of the field verification of the sampling design.

HIGHLIGHT 4-3
Elements of a QAPP

(1) Project description
(2) Designation of QA/QC responsibilities
(3) Statistical tests and data quality objectives
(4) Sample collection and chain of custody
(5) Sample analysis
(6) System controls and preventive maintenance
(7) Record keeping
(8) Audits
(9) Corrective actions
(10) Quality control reports

To the extent that potential field problems can be anticipated, contingency plans also should be specified in the SAP. An example of a contingency plan is provided in Steps 5 and 6 (Examples 5-2 and 6-1).

4.5 SCIENTIFIC/MANAGEMENT DECISION POINT (SMDP)

The completion of the ecological risk assessment WP and SAP should coincide with an SMDP. Within this SMDP, the ecological risk assessor and the ecological risk manager agree on: (1) selection of measurement endpoints; (2) selection of the site investigation methods; and (3) selection of data reduction and interpretation techniques. The WP or SAP also should specify how inferences will be drawn from the measurement to the assessment endpoints.

4.6 SUMMARY

At the conclusion of Step 4, there will be an agreement on the contents of the WP and SAP. As noted earlier, these plans can be parts of a larger WP and SAP that are developed to meet other remedial investigation needs, or they can be separate documents. When possible, any field sampling efforts for the ecological risk assessment should overlap with other site data collection efforts to reduce sampling costs and to prevent redundant sampling.

The WP and/or the SAP should specify the methods by which the collected data will be analyzed. The plan(s) should include all food-chain-exposure-model parameters, data reduction techniques, datainterpretation methods, and statistical analyses that will be used.