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Human health risk screening due to consumption of fish contaminated with chemical warfare agents in the Baltic Sea

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ABSTRACT

Chemical warfare agents (CWAs) have been disposed of in various fashions over the past decades. Significant amounts of CWA, roughly 11,000 ton, have been dumped in the Baltic Sea east of the island Bornholm following the disarmament of Germany after World War II. This has caused concerns over potential human and environmental health risks, and resulted in restrictions on fishing in the dumpsite area. The purpose of this paper is to assess the potential indirect human health risks due to consumption of CWA-contaminated fish from the dumpsite area east of Bornholm. Earlier studies suggest that the fish community may be at risk from CWA exposure in the Bornholm basin. Moreover, elevated frequencies of lesions on fish caught in a CWA dumpsite in the Mediterranean Sea have been observed. The fish at the Mediterranean dumpsite had elevated total arsenic (As) concentrations in their tissue, and elevated total As levels were also observed in the sediment. Elevated total sediment As concentrations have also been recorded in CWA dumpsites in the Skagerrak and the Baltic Sea. Triphenylarsine and sulfur mustard gas (Yperite) are the CWAs with the greatest indirect human health risk potential. There are recognized uncertainties concerning Yperite's and CWA-derived arsenical's fate and speciation in the environment, as well as their inherent toxicity, warranting caution and further site-specific environmental and human health risk assessments of CWAs dumped in the Bornholm basin.

1. Introduction

As a result of the disarmament of Germany following the Second World War, approximately 65,000 ton of stockpiled Chemical Warfare Agent (CWA) munitions was ordered by the allied forces to be disposed of. A significant portion of these were subsequently dumped at sea during the late 1940s [1,2]. The Bornholm basin in the Baltic Sea alone received more than half of Germany's CWA arsenal, comprising approximately 11,000 ton of active CWA chemical substances [1]. This is one of the worst marine CWA exposure scenarios in western Europe [3].

The munitions have been resting on the seabed and in the sediment of the Baltic Sea for approximately 60 years and the extent of corrosion of the shells, and thus release, of the toxic chemicals raise environmental and human health concerns among neighbouring Baltic countries [4]. Some shells will have leaked their content whereas others may still be intact [1,4,5]. Indirect human health risk assessment relating to fish consumption has been constrained by a lack of comprehensive understanding of the environmental concentrations of the CWA compounds disposed of at the dumpsite east of Bornholm [6]. Only one positive measured CWA value (Clark I) is currently publicly available for the Bornholm dumpsite [7]. Part of the reason for the lack of monitoring is that CWAs are illicit compounds; hence availability of chemical reference standards for analytical method development is limited. Moreover, for the same reason, until recently there were large data gaps in compiled information available on the physico-chemical properties [8], and ecotoxicities of CWAs [9]. Furthermore, there is significant uncertainty about where in the Bornholm basin the CWAs were actually dumped. There was a designated area (primary dumpsite) where all the CWAs were supposed to have been dumped; however, it is recognized that the munitions most likely were dumped in a wider area (secondary dumpsite) [1]. Hence, conservative modelled exposure estimates are required as a first step in an integrated, tiered human and environmental risk assessment.

Sanderson et al. [6], based on existing information concerning dumped CWA amounts [1,4] and on conservative model predictions, demonstrated that the fish community at the Bornholm dumpsite could potentially be at risk from CWA exposure. Earlier studies by Amato et al. [10] found significantly higher frequencies of histological lesions recorded in the Mediterranean in fish species

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from a CWA dumpsite, compared with a reference site, indicating a chronic state of illness. This presumably resulted from exposure to blistering organoarsenic CWAs, as the total arsenic (As) concentration in sediment and fish were also above reference site As levels. There is, moreover, a significant overlap between the CWA dumpsite east of Bornholm, fertile fishing grounds, and cod (*Gadus morhua*) breeding grounds, suggesting that this economically and ecologically important fish species in the Baltic Sea might be particularly exposed to dumped CWAs. These findings warrant further analysis of potential human health risks from eating fish caught in and around the dumpsite in the Bornholm basin. There are fishing restrictions within the CWA dumpsites, and still, over the past decades local fishermen have caught roughly 700 unexploded and corroded CWA bombs in their nets [11]. Some fishermen have been severely harmed by direct contact with the CWAs, typically the blistering mustard gas (Yperite), resulting in chronic illness and medical attention for years following the accidental exposure. Occupational hazards are however not evaluated in this analysis.

Before committing extensive investment in a site-specific risk assessment we recommended a tiered approach with a conservative model-based screening of potential risks to help prioritize potential subsequent site-specific assessments. This is especially important in situations where the exposure both to highly toxic compounds and potential effects are uncertain, and where it is difficult to obtain representative samples due to the scale of the potentially contaminated marine site (>100 km^2, and at depths up to 105 m), as in the Bornholm basin.

The aim of this paper is to provide a conservative model-based assessment of the predicted human health risks from eating fish from the Bornholm basin dumpsite area. This model is based on methods and approaches from the European Technical Guidance Document in support of Commission Directive 93/67/EEC on Risk Assessment for New Notified Substances, Commission Regulation (EC) No. 1488/94 on Risk Assessment for Existing Substances, Directive 98/8/EC of the European Parliament and of the Council concerning the placing of biocidal products on the market [12]. These approaches are applied to existing data on parent CWAs, model-based estimates and, when available, measured CWA property values. The nature of a conservative screening level analysis is to protect against false negatives and not against false positives. Potential false positives should be evaluated empirically in site-specific analyses if risks cannot be ruled out on the basis of a conservative and worst-case screening analysis. The total risk profile for the mix of substances will also be assessed assuming additivity of the components by adding the individual risks together [13]. It should be noted that as fishing restrictions (no fishing in the primary dump site and recommendations against fishing in the secondary dump site area) currently operate in the Bornholm CWA dumpsite area, this analysis provides a screening level scientific evaluation of the appropriateness of these.

2. Materials and methods

2.1. Compounds

Information on the quantities of parent CWAs dumped varies somewhat according to source [3]. In this assessment we rely on internationally agreed reports from the Helsinki Commission (HELCOM) [1,4].

A total of eight active CWA compounds and one additive compound have been reported to have been dumped east of the Danish island of Bornholm in the Baltic Sea [1,4,6] (Table 1), see Sanderson et al. [6,9] for individual CWAs physico-chemical properties.

Table 1
Confirmed dumped chemical warfare agents in Bornholm basin [1]

Compound	CAS number	Dumped CWA (tons)
Chloroacetophenone (CAP)[a]	532-27-4	515
Sulfur mustard gas (Yperite)[b]	505-60-2	7027
Adamsite[c]	578-94-9	1428
Clark I[d]	712-48-1	711.5
Triphenylarsine[d]	603-32-7	101.5
Phenyldichloroarsine[d]	696-28-6	1017
Trichloroarsine[d]	7784-34-1	101.5
Other (e.g. Zyklon B)[e]	74-90-8	74
Monochlorobenzene[f]	108-90-7	1405

[a] Riot control agent.
[b] Blistering agent.
[c] Organoarsenic blistering agent.
[d] Arsine oil constituents—organoarsenic blistering agent.
[e] Blood agent.
[f] Additive.

2.2. Dumpsites

The dumpsites are located in the Bornholm basin, east of the island of Bornholm, in the Baltic Sea. The primary, i.e. designated, dumping was conducted in a circular area with a radius of three nautical miles, with centre coordinates at 55°E21′′N and 15°E37′02′′E and covering an area of 97 km^2. The water depth in this location ranges from 70 to 105 m. However, not all CWA was dumped at the designated site; hence a secondary, and more realistic dumpsite is located at 55°10′′N to 55°23′′N and 15°24′′E to 15°55′′E, covering 791 km^2. The waters in the Bornholm basin can be divided into an upper and a lower layer. The upper layer (0–50 to 70 m from the sea surface) consists primarily of brackish water flowing in from the northern and eastern parts of the Baltic Sea, with a salinity of 8.1‰. This water continues to flow slowly out of the Baltic Sea into the North Sea. The lower layer (<20 m above sediment) originates in the North Sea and, on its way to the Bornholm basin, is mixed with water from the upper layer, resulting in salinity between 9.1 and 23.1‰ [1] (Fig. 1).

2.3. Modelled parent CWA water concentrations at dumpsites

The release and exposure of CWA into the marine environment and biota are influenced by several site- and compound-specific properties. We base the predicted quasi steady-state water concentrations on the findings by Sanderson et al. [6], where we assume: (1) a continuous and homogenous release, over 60 years, of the total CWA mass from the bombshells on the seabed to the water phase (the total CWA mass is homogenously distributed on either the primary (worst case) or secondary (more realistic) dumpsite areas is considered); and (2) a weak south-easterly bottom water current of 5 cm/s and vertical dispersion coefficient of 0.2 cm^2/s [14], which induces a turbulent mixing of the bulk water and an advective transport of agents. Moreover, sedimentation, diffusion to sediment, degradation (hydrolysis) and accumulation in sediment are also included in the calculations [6]. The maximum concentration predicted at 20 cm above the seafloor is in principle directly above the sediment, whereas the maximum concentration 20 m above the sediment is observed 30 km east of the dumpsite boundary, which is the longest straight line transport trajectory before a circular motion is initiated [15] (Table 2). These values are used to derive the CWA external exposure distributions for fish in water based on the compounds' bioconcentration factors (BCF) [9] and biomagnification factors (BMF) [12]. CWA levels in fish can thus be derived and an indirect human health screening level assessment of risks can be performed in accordance with the EU TGD Part 2, Chapter 4 on human health risk assessment [12].

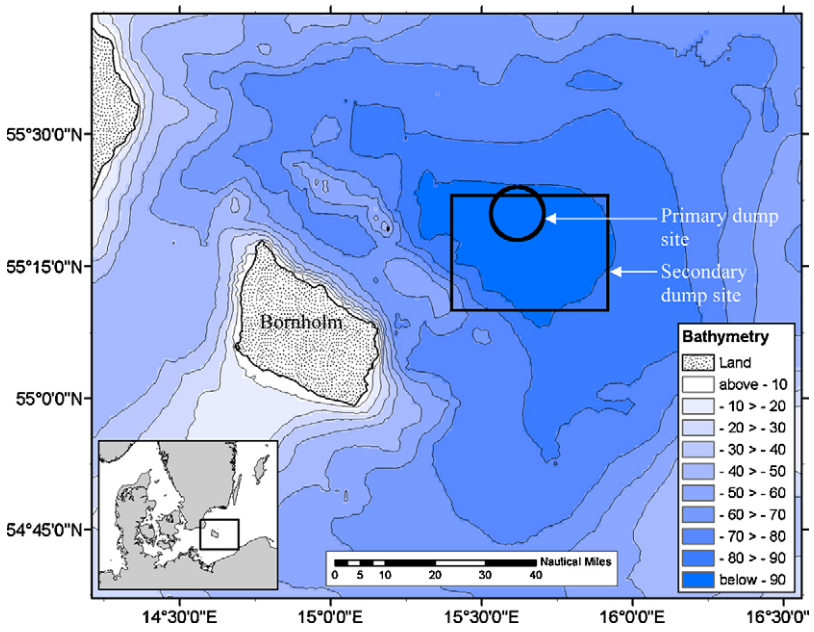

Fig. 1. Map of primary and secondary dump site, Bornholm basin.

2.4. Human health risk screening methods

Here, we only consider oral exposure via consumption of contaminated fish. For the overall assessment and appreciation of potential human health hazards (primarily mutagenicity and carcinogenicity) we used the Danish (Q)SAR database (http://ecbqsar.jrc.it/oasis/dbstart.html?enterButton=Start+search) in combination with the U.S. National Library of Medicine under the U.S. National Institute of Health (NIH) toxicology data network database on hazardous substances (http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB), as reported in Sanderson et al. [6], plus the Agency for Toxic Substances and Disease Registry (ATSDR) (http://www.atsdr.cdc.gov/). To derive reference doses for chronic oral exposure (RfD) we used the U.S. Environment Protection Agency Integrated Risk Information System (EPA IRIS) (http://cfpub.epa.gov/ncea/iris/index.cfm) and Opresko et al. [16]. The RfD concentrations are assumed on the basis of all known facts not to result in any harm towards humans. The resulting screening level human health risk equation (Eq. (1)) based upon EU TGD [12] is thus:

$$\text{Risk value} = \frac{\text{RfD}_{\text{oral}}(\text{mg/kg BW/day})}{\text{Waterconc(mg/L)}\times\text{BCF}\times\text{BMF}\times 70(\text{kg BW})\times 0.115(\text{kg fish/day})} \quad (1)$$

where RfD is the oral reference dose for each CWA; water concentration is the predicted concentration for each CWA; BCF is the bioconcentration factor for each compound; BMF is the biomagnification factor (BMF = 1 for all CWA materials ($\log K_{ow} > 4.5$ and BCF > 2000), except for triphenylarsine ($\log K_{ow} = 5$–8, and/or BCF > 5000 yielding a BMF of 10)) [12]; EU default standards: 70 kg BW, and 0.115 kg fish consumed/day/person [12].

As the water concentration is variable in both time and space the concentration value in the equation must be interpreted as an effective average concentration surrounding the fish. The human health margin of exposure (MOE) is the reciprocal value of the human health risk value (MOE = 1/risk value).

Oral reference doses were obtained from the EPA IRIS database for monochlorobenzene and organoarsenic CWAs, with inorganic arsenic (CAS# 7440-38-2) as proxy for the organoarsenic CWAs as recommended by Munro et al. [8] and the U.S. National Institute of Health. Zyklon B oral RfD was derived from (http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB). It should be noted that inorganic arsenicals are the highest ranked priority toxicant on the US Agency for Toxic Substances and Disease Registry (http://www.atsdr.cdc.gov/) list of 250 priority toxic substances. Oral RfD for Yperite was derived from US Agency for Toxic Substances and Disease Registry (ATSDR) (http://www.atsdr.cdc.gov/toxprofiles/tp49.pdf) and Opresko et al. [16]. The lowest reported oral effect value for CAP was chosen and an uncertainty factor of 1000 was applied to derive an approximate oral RfD. Oral mammalian lethal effect concentrations for CAP were obtained from the U.S. National

Table 2
CWA predicted water concentrations (mg/L) at 20 cm and 20 m above the sediment surface[a] [6]

Compound	Concentration lower water layer (20 cm)		Concentration upper water layer (20 m)	
	Primary dump site	Secondary dump site	Primary dump site	Secondary dump site
CAP	3E-5	6E-6	8E-9	1E-9
Yperite	4E-4	8E-5	1E-7	1E-8
Adamsite	8E-5	2E-5	2E-8	3E-9
Clark I	4E-5	9E-6	1E-8	1E-9
Triphenylarsine	5E-6	1E-6	1E-9	1E-10
Phenyldichloroarsine	6E-5	1E-5	1E-8	2E-9
Trichloroarsine	6E-6	1E-6	1E-9	2E-10
Other (Zyklon B)	4E-6	6E-7	3E-10	3E-11
Monochlorobenzene	7E-5	1E-5	7E-9	9E-10

[a] The predicted concentrations at the sea surface are negligible (<1E-16 mg/L) for all agents.

Table 3
Predicted fish CWA concentrations based on realistic worst-case exposure scenarios (mg/L)

Compound	BCF	Fish external water concentration (mg/L)		Fish internal tissue concentration (mg/kg)	
		Primary dump site	Secondary dump site	Primary dump site	Secondary dump site
CAP	0.8	1E-5	3E-6	1E-5	2E-6
Yperite	14.3 (.3)[a]	2E-5	4E-5	3E-4	6E-5
Adamsite	262	4E-5	9E-6	1E-3	2E-4
Clark I	600	2E-5	4E-6	1E-3	3E-4
Triphenylarsine	7901	3E-6	5E-7	0.2	0.04
Phenyldichloroarsine	45.6	3E-5	6E-6	1E-4	3E-5
Trichloroarsine	3.5	3E-6	6E-7	1E-5	2E-6
Other (Zyklon B)	3.2	2E-6	3E-7	6E-6	9E-7
Monochlorobenzene	30.7	4E-5	6E-6	1E-4	2E-5
TOTAL CWA mixture	–	4E-5	7E-5	0.23	0.045

[a] Measured fish BCF value in brackets [19].

Library of Medicine under the U.S. National Institute of Health toxicology data network database over hazardous substances (http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB (Table 4).

3. Results

3.1. Environmental CWA exposure and predicted fish concentrations

From confirmed information concerning amounts of CWA dumped (Table 1) and the methods developed in Sanderson et al. [6], modelling allows calculation of conservative predicted exposure concentrations. This assumes a continuous release over 60 years and simple first-order dissipation, and that the entire CWA tonnage was dumped in either the primary dumpsite or the larger and more likely secondary dumpsite (Table 2).

The commercially relevant fish species from the Bornholm basin is primarily cod (*G. morhua*), and to a lesser extent herring (*Clupea harengus*) and sprat (*Sprattus sprattus*). The peak density of these three species, and where cod eggs float, is at a depth of 60–70 m; at greater depths oxygen levels in the Bornholm basin typically approach the oxygen threshold for these species (<3 mg/L) [17]. However, cod will forage at greater depths with close to anoxic conditions in swift 'round trips' [18]. In this analysis we thus conservatively approach a realistic worst-case exposure scenario, assuming that a fish will be present for 95% of its lifetime at a depth of 70 m (20 m above the seafloor) and 5% at 90 m (20 cm above the seafloor); hence the fish CWA external exposure concentration weighted according to behavioural patterns is a lifetime average:

$$C_{\text{fish external exposure}} = 0.05C_{\text{lower water layer}} + 0.95C_{\text{upper water layer}} \quad (2)$$

where $C_{\text{fish, exposure}}$ is the weighted CWA exposure concentration around a fish; $C_{\text{lower water layer}}$ and $C_{\text{upper water layer}}$ represent the figures given in Table 2.

The resulting CWA concentration in fish tissue is thus the weighted external fish water exposure from (Eq. (2)) multiplied by the BCF and the BMF of each CWA for both the primary and secondary dumpsites (Table 3):

$$C_{\text{fish internal}} = C_{\text{fish external exposure}} \times \text{BCF} \times \text{BMF} \quad (3)$$

where $C_{\text{fish external}}$ is calculated via Eq. (2); BCF is given in Table 3; BMF = 1 for all CWAs, except triphenylarsine (BMF = 10) (see Eq. (1)).

3.2. Human health toxicity profiles of CWAs

It is of course evident that chemical warfare agents are toxic towards humans. However, these substances have primarily been developed and tested in relation to producing harmful effects via human dermal exposure and inhalation; hence oral exposure, a secondary exposure route relevant in terms of fish consumption, has been examined to a lesser extent. A qualitative screening of CWA toxicological profiles revealed that Yperite is cytotoxic (it is used as an antineoplastic drug), mutagenic, and carcinogenic; organoarsenic CWAs (inorganic As surrogate) are mutagenic and carcinogenic; CAP is not mutagenic but is a known carcinogen, while monochlorobenzene is mutagenic in mouse lymphoma and micronucleus tests. Zyklon B has not been measured or predicted to be either mutagenic or carcinogenic [6,16].

Table 4
CWA oral reference dose (RfD) (mg/kg BW/day)

Compound	CAS number	Oral RfD (mg/kg BW/day)
CAP	532-27-4	5E-3
Yperite	505-60-2	7E-6
Adamsite	578-94-9	3E-4
Clark I	712-48-1	3E-4
Triphenylarsine	603-32-7	3E-4
Phenyldichloroarsine	696-28-6	3E-4
Trichloroarsine	7784-34-1	3E-4
Other (Zyklon B)	74-90-8	2E-3
Monochlorobenzene	108-90-7	2E-3

3.3. CWA human health risk profiles and margins of exposure

Combining the calculated indirect consumer exposure via fish consumption from Table 3 with the RfDs from Table 4 a worst-case screening level human health risk value and the corresponding margin of exposure are obtained by using Eq (1), in accordance with the EU TDG [12] for the primary and secondary dumpsites (Table 5).

The results in Table 5 indicate that there is a potential risk to fish consumers, with conservative total MOEs ranging from 0.52 to 2.63 according to the respective exposure scenario (primary versus secondary dumpsite). It is also clear that triphenylarsine is the

Table 5
Human health risk values and margin of exposures

Compound	Human health risk value		Margin of exposure (MOE)	
	Primary dump site	Secondary dump site	Primary dump site	Secondary dump site
CAP	4E-7	8E-8	3E6	1E7
Yperite	0.67	0.14	1.5	7.2
Adamsite	6E-3	1E-3	17	80.6
Clark I	7E-3	1E-4	15	70.4
Triphenylarsine	1.1	0.21	0.9	4.7
Phenyldichloroarsine	7E-4	1E-4	140	702
Trichloroarsine	5E-5	1E-5	2E4	9E4
Other (Zyklon B)	5E-7	8E-8	2E6	1E7
Monochlorobenzene	9E-5	2E-5	1E4	6E4
TOTAL CWA mixture	1.91	0.37	0.52	2.63

CWA with the greatest risk potential followed by Yperite and the organoarsenic CWAs; Clark I, and Adamsite, respectively (with inorganic As as RfD proxy). The US EPA [13] fish advisories' limits for monthly fish consumption with regard to inorganic arsenic would allow a maximum of eight fish meals per month (1 in 100,000 risk level) for non-cancer health endpoints based on inorganic As as a proxy for the maximum total CWA concentration in fish tissue (0.231 mg/kg) for the primary dumpsite (Table 3). There would be no restrictions on fish meals per month for the secondary dumpsite scenario as the total CWA is <0.04 mg/kg here for non-cancer health endpoints. However, for cancer health endpoints the US EPA [13] would recommend zero fish meals per month for the primary dumpsite, and a maximum of one meal per month for the secondary dump site. The BMF of 10 for triphenylarsine drives the organoarsenic CWA exposure. There are no advisories regarding Yperite.

4. Discussion

The dumped Yperite is estimated to contain 80% normal Yperite and 20% viscous Yperite (also known as winter-Yperite, mixed with arsenic oils and polymers). Viscous Yperite is more persistent but also less bioavailable than normal Yperite. Therefore, we assume, at a conservative screening level, that both forms with time will be bioavailable. In 1997 a 5 kg block of winter-Yperite was caught in the Baltic Sea by Polish fishermen, leading to hospitalization of four members of the crew. The analysis of a clammy and grease-like Yperite block revealed 50 different compounds. The majority (14–20%) of the total mass was Yperite; significant amounts of Clark I were also recorded. The major hydrolysis product of Yperite, thiodiglycol, was not present in the block, presumably because it had already passed to the surrounding water [20]. Granblom [21] measured a worst-case Yperite concentration in the top 4 cm of sediment at the Skagerrak CWA dumpsite of 0.00019 mg/kg, 1 km away from a wreck, suggesting that Yperite can both persist and move in the environment. Yperite is, as a mentioned above, a carcinogen that acts as a bifunctional alkylating agent, reacting rapidly with nucleophiles in the body via the intermediate episulfonium ion. The human nucleophilic species includes DNA, and the primary site of DNA alkylation by Yperite is the N7 position of deoxyguanosine residues. The major human metabolites of Yperite are also thiodiglycol and thiodiglycol sulfoxide. Roughly 50–90% of Yperite is excreted in this form by mammals in the urine within 24 h, while the remaining part of Yperite and its metabolites can persist in the blood for weeks to months [22]. It is the chronic toxicity (carcinogenicity) that yields Yperite's low oral RfD (Table 4). The corresponding oral RfD for the metabolite, thiodiglycol, is roughly 10,000 times higher than Yperite at 0.4 mg/kg BW/day [23]. The Danish authorities reported a measured bioconcentration factor for normal Yperite in 1986 of 0.3 in the marine fish species, plaice (*Pleuronectes* sp.) [19]. The resulting fish tissue concentrations of Yperite with a BCF of 0.3 would thus be 6E-5, and 1E-5 mg/kg, for the primary and secondary dumpsites, respectively. Considering the measured BCF value of 0.3, instead of the predicted worst-case BCF of 14.3 (Table 4), the resulting Yperite MOEs would be 71 and 341, and a total CWA mixture MOEs would be 0.8 and 4.1 for the primary and secondary dumpsites, respectively.

It has been argued that at temperatures at <5 °C the solubility of the CWA compounds decreases significantly, leading to a HELCOM preliminary conclusion that Adamsite (and other organoarsenic CWAs) and Yperite are insoluble in seawater and that they will remain as solid brittle lumps (Adamsite) or jelly-like lumps (Yperite) at the seabed, supposedly precluding bioavailability and not causing any risk [4]. From a precautionary point of view, both from a human health and an environmental risk perspective, this conclusion for the potentially most problematic CWAs dumped at sea, in a complex environment over long time spans has been challenged by Sanderson et al. [6], and warrants further analysis. The assumption of zero exposure and bioavailability contradicts the findings of Amato et al. [10], who found both elevated lesion frequencies in fish and elevated total As concentrations in sediment and fish from a CWA dumpsite compared with reference conditions. Furthermore, Tørnes et al. [5] and Garnaga et al. [7] also reported elevated As concentrations in dumpsites in the Skagerrak and in the Baltic Sea compared to reference conditions, respectively, suggesting that dumped CWAs can yield locally increased As contamination. It is widely accepted that degradation of organoarsenic CWAs can lead to elevated As contamination relative to reference conditions [5,7,8,10,24], however, the subsequent speciation of As in sediment [5,24], water, and biota (fish) is uncertain. Tørnes et al. [24] found an average CWA-derived total As concentration of 48.4 (±190 S.D.) mg As/kg (dw) in the top 3 cm of the sediment in the Skagerrak CWA dumpsite. Clark I derivates were the highest contributors with an average of 63.2 (±220 S.D.) mg/kg (dw). The average for triphenylarsine was 7.3 (±15.6 S.D.) mg/kg (dw). Peak arsenical concentrations were observed close to wrecks [5,24]. Amato et al. [10] found 44.8 mg As/kg (dw) in sediment in a CWA dumpsite. Background concentrations in the area were close to 5 mg As/kg (dw) in sediment. The only publicly available organoarsenic CWA finding reported for the Bornholm dumpsite to date is 10 mg Clark I/kg sediment, recorded from the secondary dumpsite. Anomalies in the total As concentrations ranging from 18 to 210 mg As/kg sediment (average = 25 mg As/kg) were also reported for the Bornholm dumpsite [7]. Simple linear equilibrium partitioning calculations [6] based upon the measured 10 mg Clark I/kg sediment in the Bornholm dumpsite yield 1.1E-3 mg Clark I/kg fish tissue versus the predicted 1.2E-3 mg Clark I/kg fish tissue.

The speciation of As in fish [25] is of critical importance in relation to the potential toxicity and risk evaluation of organoarsenic CWAs [26,27]. The valency of inorganic As as either trivalent or pentavalent is important, as compounds containing the trivalent form are in general far more toxic than those in the penta form [26,28]. On ingestion, As is metabolized from inorganic to organic compounds. The metabolization also involves reduction of pentavalent As to the trivalent state (which initially increases the toxicity) and subsequent hepatic biomethylation to form monomethyl arsenic (MMA), dimethyl arsenic acids (DMA), and trimethylarsine oxide (TMAO). Overall these steps reduce the acute toxicity of the As significantly. However, DMA has been shown to be a tumour promoter, and MMA has been shown to be genotoxic *in vitro* [26,27,29]. Reactive oxygen species (ROS) are also thought to play a significant role in trivalent As species' toxicity and disruption of DNA synthesis and repair. Most of the neurotoxic effects and neuropathy caused by As resemble the Guillain-Barré syndrome and are caused by inactivation of enzymes in the cellular energy pathway [26].

Approximately 75–90% of total As in the human diet, according to the Danish Food Agency, comes from eating seafood, with a total As intake for the average Danish person of 118 μg/day [30]. It is estimated that only 1% of the total As intake is in the more toxic inorganic form. The permissible level of inorganic arsenic in edible fish tissue is 6 μg/kg [31], and it should be noted that arsenic is a naturally occurring element that is ubiquitous to ocean water, with typical levels at 1–2 μg/L [32]. Amato et al. [10] found 29.7 mg total As/kg fish (dw) from the CWA dumpsite, whereas fish under the reference conditions had total As levels that were more than 10 times lower at 1.9 mg/kg (dw). Cod has the highest total As levels with a maximum of 11.5 mg As/kg (ww) among fishes from Danish waters, including the Bornholm basin [30]. The majority of total As in fish is in the form of arsenobetaine (35–100%), with 7–62% DMA and 4–14% TMAO as the other larger constituents [25]. Spe-

ciation of total As in fish from CWA dumpsites has so far not been assessed. Only 1–4% of the total As in fish is in the inorganic form [25,30,31]. In a more recent review of As speciation in fish and seafood, Schoof and Yager [31] found that 3.9% of the total As in marine fish is comprised of DMA. The largest As constituent in fish, arsenobetaine, is largely inert, non-toxic ($LD50_{oral\ mouse}$ = 10 g/kg) and rapidly excreted representing the end station of the As cycle in the marine ecosystem. Arsenobetaine can be degraded by microorganisms but is generally not transformed in humans and is excreted essentially unchanged [32]. Arsenicals in fish bioconcentrates significantly in liver and intestines, and only arsenobetaine has been reported in fish muscle (fillet) [25,31]. Hence, from a pragmatic risk assessment perspective one can assume that 1–4% of total As in fish is in the toxic inorganic form [30,31].

In summary we can assume in a worst-case scenario that 4% of total predicted organoarsenic CWA predicted in fish is highly toxic. This increases the organoarsenic CWA MOE by 25-fold, yielding values of 20 and 104 depending upon the respective exposure scenario (primary versus secondary dumpsite), suggesting lower indirect human health risks from consuming fish that is potentially exposed to organoarsenic CWAs in the Bornholm basin. The organoarsenic CWA MOE can even be increased by a factor of 3, as the ratio between inorganic As to ultimately degraded organoarsenic CWA mass is roughly 1:3, yielding MOEs ranging from 60 to 300.

Assuming a similar speciation of CWA-derived As in other sources in fish and that consumers only eat the fish muscle (fillet), the potential risk would be negligible as toxic forms of As have not been detected in fish muscle [25]. Moreover, it is unlikely that cooking arsenobetaine-containing fish will generate toxic As species at clinically relevant levels [31,32]. However, since ingestion of seafood with different species of As may lead to generation of metabolites (DMA) involved in arsenic-induced carcinogenesis, it is worth considering the role of dietary seafood in long-term cancer risk scenarios [32]. Generally, a site-specific assessment is required to evaluate risks from chronic exposures to toxic forms of As via fish consumption. However, considering the potency and nonlinearity of DMA carcinogenicity it appears that DMA in seafood will make a negligible contribution to any cancer risk associated with As in fish [31]. Others find that arsenicals, including DMA, may be more mutagenic, cytotoxic and carcinogenic than inorganic As [33]. In conclusion, arsenicals are paradoxical substances as they are the only compounds for which the International Agency for Research on Cancer (IARC) considers carcinogenic for humans despite inadequate evidence for animal carcinogenicity. The mechanisms are still widely unknown, impeding thorough and accurate epidemiological studies of these compounds [34]. Caution is thus warranted, and it is important to note the uncertainties and knowledge gaps in terms of arsenicals carcinogenesis [27,29,34]. In addition the fact that As is ATSDR's (http://www.atsdr.cdc.gov/) top-priority compound in a risk assessment context underscores the need for caution.

If the organoarsenic CWAs were ignored in the overall CWA mixture risk analysis due to lack of human indirect exposure via consumption of fish fillet, Yperite alone would result in MOEs of 1.5 (71) and 7.2 (341) for the two dumpsites. The MOEs based on the measured BCF of 0.3 in brackets suggests a potential risk under conservative worst-case assumptions. Monitoring the potential indirect human health risk from Yperite would challenge current analytical methods, as limits of detection (LOD) in fish tissue should be <7 parts per trillion (10^{-9}) or 7 ng/kg in order to detect safe exposure levels below the oral RfD for Yperite. Hence, due to inherent uncertainties and the ranges of the MOEs being dependent upon assumption scenario, further empirical site-specific risk assessment of CWAs dumped in the Bornholm basin with focus on Yperite and organoarsenic CWAs, as covered in this screening level assessment, is warranted. There is no conclusive evidence to support relaxation of the current fishing restrictions in the Bornholm CWA dumpsites.

5. Conclusions

- The screening level assessment does not suggest the fishing limitations in the dumpsites be relaxed.
- Triphenylarsine and Yperite are the compounds associated with the greatest risk; triphenylarsine due to its physico-chemical properties and Yperite due to the large amounts dumped and its high chronic toxicity (carcinogenicity) and thus low oral RfD. Other organoarsenic CWAs are also potentially associated with risk because of their relatively high BCF and low oral RfD due to the use of inorganic As toxicity values as proxies for them.
- Under a worst-case scenario consumption with respect to cancer health endpoints, a maximum of 0–1 fish meals per month caught from the primary and secondary dumpsites, respectively, is recommended, based on the presence of organoarsenic CWAs alone. There are no recommendations regarding other CWAs, including Yperite.
- The above conclusions should be evaluated more carefully in an empirical site-specific risk assessment. There are uncertainties concerning human exposure to Yperite and speciation of As in the environment and in fish from a CWA dumpsite, as well as the carcinogenesis of arsenicals.

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