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Estimation of dietary intake and human health risk of hexachlorobenzene by marine organism consumption in Korea

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Hexachlorobenzene (HCB) was analyzed in various marine organisms of Korea. HCB was detected in all organism samples with residual concentrations from 0.51 to 222 pg/g wet weight. HCB residue was the highest content in crustacean, and followed by bivalves, fish, cephalopods and gastropods. The residues were comparable to or lower than those in marine organisms of other countries. Daily dietary intake of HCB from seafood was estimated to be 13.4 pg/kg body weight/day. The relative contribution of taxonomic group to the total dietary intake of HCB were in the order of crustaceans (40.1%), bivalves (34.2%), fish (23.1%), cephalopods (2.22%), and gastropods (0.38%). Daily dietary intake of HCB expressed as toxic equivalent (TEQ) value was estimated to be 1.34×10^{-3} pg TEQ/kg body weight/day. This value did not exceed tolerable daily intake (TDI) proposed by the WHO, the UK toxicity committee and the KFDA. Cancer risk and target hazard quotient (THQ) due to the consumption of the marine organism in Korean adult population were evaluated using the exposure equation of food ingestion. This result suggests that dietary intake of HCB by the consumption of Korean seafood seems to be safe for human health with negligible cancer and non-cancer risks so far.

Key Words : Hexachlorobenzene (HCB), Dietary intake, Cancer risk, Target hazard quotient (THQ), Human health

1. Introduction

Persistent organic pollutants (POPs) are organic chemical compounds that are highly toxic, persist in the environment, bio-accumulate in fatty tissues of living organisms, travel long distances, and naturally migrate toward colder climates[1]. The twelve POPs designated as targets for early global action are all chlorine-containing organic compounds including polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), polychlorinated biphenyls (PCBs), and organochlorine pesticides (OCPs).

Hexachlorobenzene (HCB) among these POPs has been used as both a pesticide and an industrial chemical in recent years[2,3]. While intentional production has declined, HCB is still produced as a byproduct during the manufacturing of several chlorinated chemicals. HCB has been detected in various abiotic and biotic compartments such as air[4], water[5], soil[6], sediments[7], birds[8], fish[9], adipose tissue[10], breast milk[11], and human blood[12]. HCB has shown to be carcinogenic in rodent[13], and has also been classified by the US EPA and the International Agency for Research on Cancer (IARC) as a probable human carcinogen[14,15].

An extensive survey has been launched in National Fisheries Research & Development Institute (NFRDI), to determine the presence of POPs in the marine environments of Korea. In particular, the residues of PCDDs/DFs, dioxin-like PCBs, OCPs, polycyclic aromatic hydrocarbons (PAHs) and tributyltin (TBT) in various marine organisms have been determined. The results on dietary intakes and human health risks of PCDDs/DFs[16], dioxin-like PCBs[16], PAHs[17], and TBT[18] have been reported.

Seafood is probably the primary source of protein for people in the world and an important part of diet in Korean population. People can be exposed to toxic chemicals through the contaminated seafood. Actually,

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the exposure of humans to toxic contaminants occurs mainly (>95%) through the contaminated food[19,20]. The other exposure pathways contribute bioaccumulation to a negligible extent[21,22]. Hence, determination of the concentration of these compounds in various marine organisms are very important for evaluation of toxic dietary exposure, particularly due to the increase of seafood consumers. The objectives of the present study are to estimate the dietary intake of HCB via various popular marine organisms in Korea and to assess human health risks by their consumption.

2. Materials and Methods

2.1. Sample collection

Thirty-five marine organisms were sampled at local fish markets in Korea from January 2002 to October 2003. These organisms are commonly consumed species and are commercially important in Korea. The selected marine organisms can be classified into 5 groups including fish (21 species), crustaceans (4 species), bivalves (6 species), gastropods (2 species), and cephalopods (2 species). Biological information on these marine organisms were summarized in Table 1.

2.2. Sample preparation and instrumentation

Marine organism samples were stored in a cooler box with ice or dry ice and immediately transported to the laboratory. The muscles of fish and cephalopod were homogenized with an ultra-disperser. The shells of crustaceans, bivalves and gastropods were removed and the whole soft tissues were pooled and homogenized. Samples were stored in -20℃ before extraction. Internal standards ($^{13}C_6$-HCB, Wellington Laboratories, Canada) were spiked into individual homogenized samples (approximately 25 g) and were then digested in 150 mL of 1 N KOH ethanolic solution for 2 hours by mechanical shaking. The digest was liquid-liquid extracted twice using 150 mL of *n*-hexane (Ultra residue analysis, J. T. Baker) after the addition of water and 50 g of anhydrous Na_2SO_4. The extracts were reduced to a small volume in a rotary evaporator and then adjusted to a volume of 10 mL.

Marine organism samples were cleaned up a multilayer silica based adsorbent (70-230 mesh, Neutral, Merck) column (15 mm inner diameter, 300 mm length) with 120 mL of *n*-hexane. The elution flow was set at 10 mL/min. The extract was passed through adsorbents in the following order; anhydrous sodium sulfate 3 g, silver nitrate impregnated silica gel 2 g, silica gel 0.4 g, 22% sulfuric acid impregnated silica gel 2 g, 44% sulfuric acid impregnated silica gel 2 g, silica gel 0.4 g, 2% potassium hydroxide impregnated silica gel 1 g, and finally silica gel 0.4 g. The purified samples were concentrated to less than 1 mL, and left at a room temperature for one day to evaporate to 50-100 μL. The residues were dissolved with 50 μL of *n*-nonane (Pesticide residue analysis, Fluka, Switzerland) and analyzed for HCB with GC/MSD (Agilent 5973N, USA). Further details of the experimental procedure and instrumental analysis of HCB were presented in Moon et al.[23].

Procedural blanks were processed in the same manner as real samples, and they were below 10% of analytes abundance. Blanks were run before and after standards to check for carryover. Sample recoveries were in the range of 84-102%. The data presented in this investigation were not corrected for recoveries.

2.3. Calculation of dietary intake

The average body weight of the Korean adult was set as 60 kg. The data on average daily dietary intake of marine organisms were obtained from the Ministry of Health and Welfare (MOHW)[24]. Total averaged daily ingestion of foods in Korean populations was 1,315 g. Seafood was 64.1 g or 5% of total food ingestion. This value included both natural and processed seafoods. The ingestion values were calculated as the sum of all kinds of sampled natural organisms. Daily dietary intake of HCB via consumption of various marine organisms was calculated by multiplying the HCB concentrations in organism samples (pg/g wet weight basis) by the ingestion of individual seafood available (average daily intake in g/day).

3. Results and Discussion

3.1. HCB residues in marine organisms

The concentrations of HCB in various marine organisms in Korea were presented in Table 1. The moisture and lipid contents (dry weight basis) in the organisms were in the ranges of 46-84% and 2.4-48%, respectively. HCB was detected in all organism samples and the residue ranged from 0.51 to 222 pg/g wet weight.

Concentrations of HCB in fish species varied from 0.51 to 19.3 pg/g wet weight. The highest content of HCB was detected in herring (*Clupea pallasii*) from

Pohang coast, while the anchovy (*Engraulis japonica*) from Tongyeong coast showed the lowest concentration. HCB residues in curstacean species were in the range of 5.46-222 pg/g wet weight, showing the widest bioaccumulation range of HCB in comparison to other taxonomic groups. The hair crab (*Erimacrus isenbecki*) and red snow crab (*Chinooecestes japonicus*) showed higher residues of HCB than those of other species. The levels of HCB in bivalve species ranged from 8.9 to 196 pg/g wet weight. Mussel (*Mytilus coruscus*, 196 pg/g wet weight) from the Gwangyang coast and the Pacific oyster (*Crassostrea gigas*, 104 pg/g wet weight)

Table 1. Biological characteristics and hexachlorobenzene concentration (pg/g wet weight) in various marine organisms of Korea

Species	Local market	n[a]	Length (cm)	Height (cm)	Moisture (%)	Lipid[b] (%)	C[c]
Fish							
Korean flounder (*Glyptocephalus stelleri*)	Gangleung	16	22	9.5	74	4.6	1.73
Roundnose flounder (*Eopsetta grigorjewi*)	Jumunjin	12	28	10	75	13	2.11
Alaska pollack (*Theragra chalcogramma*)	Jumunjin	10	38	6.8	75	5.0	5.43
Sailfin sandfish (*Arctoscopus japonicus*)	Jumunjin	40	16	3.3	76	27	9.24
Pacific saury (*Cololabis saira*)	Pohang	11	28	3.5	70	21	4.44
Herring (*Clupea pallasii*)	Pohang	6	27	6.1	77	47	19.3
Hairtail (*Trichiurus lepturus*)	Busan	5	31	8.2	79	19	1.97
Mackerel (*Scomber japonicus*)	Busan	5	42	6.0	81	48	2.84
Anchovy (*Engraulis japonica*)	Tongyeong	60	4.2	1.3	77	12	0.51
Sea bass (*Lateolabrax japonicus*)	Yeosu	13	43	5.0	75	20	7.94
Red tongue sole (*Cynoglossus joyneri*)	Yeosu	10	24	2.4	79	3.5	3.99
Sharp toothed eel (*Muraenesox cinereus*)	Yeosu	10	33	4.2	81	14	2.23
Butter fish (*Pampus argenteus*)	Yeosu	7	19	10	81	18	1.09
Brown croaker (*Miichthys miiuy*)	Mokpo	2	85	16	80	13	1.98
Spanish mackerel (*Scomberomorus niphonius*)	Mokpo	6	70	30	76	29	5.73
Oliver flounder (*Paralichthys olivaceus*)	Gunsan	20	33	13	76	10	2.24
Rockfish (*Sebastes schlegeli*)	Incheon	2	30	10	78	14	9.29
Marbled rockfish (*Sebastiscus marmoratus*)	Jeju	25	18	6.1	77	12	2.18
Korean pomfret (*Pampus echinogaster*)	Gunsan	8	20	10	84	15	1.31
Armorclad rockfish (*Sebastes hubbsi*)	Gunsan	20	21	6.4	62	11	9.24
Gray mullet (*Mugil cephalus*)	Incheon	2	22	6.2	84	3.1	12.9
Crustacean							
Hair crab (*Erimacrus isenbecki*)	Gangleung	30	28	5.3	55	15	222
Snow crab (*Chionoecetes opilio*)	Pohang	5	55	20	72	14	25.2
Red snow crab (*Chionoecestes japonicus*)	Pohang	20	40	17	78	15	126
Blue crab (*Portunus trituberculatus*)	Incheon	10	18	10	79	2.4	5.46
Bivalve							
Mussel (*Mytilus coruscus*)	Sokcho	100	12	4.6	79	4.6	196
Pacific oyster (*Crassostrea gigas*)	Asan	80	3.4	2.4	46	10	104
Korean scallop (*Chlamys farreri*)	Jumunjin	70	7.0	1.7	67	4.2	8.90
Japanese cockle (*Fulvia mutica*)	Yeosu	50	18	7.0	71	3.0	23.6
Razor clam (*Solen strictus*)	Mokpo	70	5.8	1.2	76	2.6	29.2
Manila clam (*Ruditapes philippinarum*)	Mokpo	100	3.3	1.4	81	3.9	12.6
Gastropod							
Abalone (*Haliotis discus hannai*)	Pohang	10	10	3.2	74	2.8	8.45
Giant abalone (*Haliotis gigantea*)	Jeju	10	15	12	67	3.9	3.90
Cephalopod							
Cuttle fish (*Todarodes pacificus*)	Gangleung	8	4.3	4.3	73	4.4	3.04
Whiparm octopus (*Octopus minor*)	Mokpo	7	18	18	63	3.1	1.54

n[a]: sample numbers; Lipid[b] in the dry weight basis; C[c]: HCB concentration.

from the Asan coast contained high levels. Gastropods and cephalopods showed relatively low concentrations of HCB.

The comparison of average HCB residues in various marine species in Korea was presented in Fig. 1. Crustaceans and bivalves showed high contents and other taxa showed the low residues of HCB. The contamination tendency of HCB in various marine organisms of Korea is similar to those of PCDDs/DFs in the same organism samples[16]. This means that the main source and/or bioaccumulation character of HCB in marine organisms is similar to those of PCDDs/DFs. Kim et al.[25] reported that the contamination of HCB in the Korean marine environment might result from the byproduct deriving from combustion processes rather than the application as pesticides. Actually, HCB have not been introduced into Korea.

Fish species with a high trophic level showed a low concentration in comparison to other taxa. This is due to their high metabolic character relative to the other taxa. This indicates that HCB migrated into fish can be eliminated by biotransformation and/or biodegradation with greater efficiency[26,27]. However, there was a significant correlation (r=0.76, p<0.001) between lipid contents and HCB residues in fish (Fig. 2). Many authors reported that fish with a high lipid content showed the high bioaccumulation properties of toxic organic contaminants[28,29].

HCB residues in various marine organisms in Korea were compared with those from other countries (Table 2). HCB residues in bivalves in this study were in the range of 0.01-0.20 ng/g wet weight. This values were comparable to HCB levels detected in Korean coasts[25], Tyrrenian Sea in Italy[29], and Sri Lanka[30].

HCB residues in fish in this study were slightly lower values than those of Sri Lanka[30] and Norway[37]. However, HCB residues from other reports were greater than those in this study. In particular, HCB concentrations in fish from Tokyo Bay in Japan[31] and Egypt[33] revealed an order of about 10-1,000 times greater than those reported in this study. Consequently, HCB residues in marine organisms from Korean coasts were low or moderate level with respect to marine environments from other countries.

3.2. Dietary intake of HCB via various marine organisms

The daily dietary intakes of HCB from marine species were calculated as shown in Table 3. The dietary intake of HCB by marine organism consumption in Korea was estimated to be 12.7 pg/kg body weight/day. Daily dietary intake of HCB by fish consumption was 2.61 pg/kg body weight/day. Crustacean species were characterized by the highest exposure to HCB with 5.21 pg/kg body weight/day. Bivalve's daily intake of HCB was 4.58 pg/kg body weight/day. Gastropod and cephalopod species showed relatively low intakes of HCB.

The relative contribution of individual species to the total dietary intake of HCB showed high values in order to crustaceans, bivalves, fish, cephalopods, and gastropods by 40.9%, 35.9%, 20.5%, 2.34% and 0.4%, respectively (Fig. 3). Although fish showed the highest amount of ingestion compared to those of the other marine species, the relative contribution to total dietary intake of HCB was not so high due to their low residues. Angélique[38] suggested that HCB should be clas-

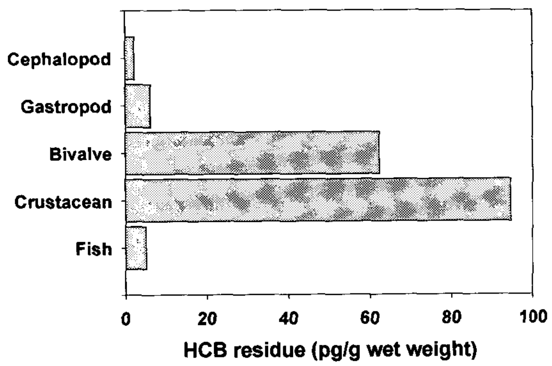

Fig. 1. Comparison of average HCB residues in various marine organisms of Korea.

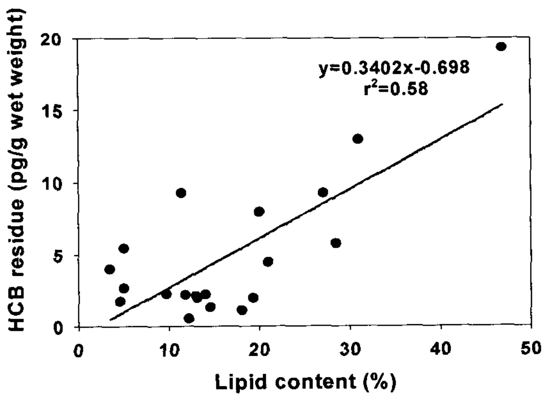

Fig. 2. Relationship between lipid contents and HCB residues in fish of Korea.

Table 2. Comparison of HCB concentrations (ng/g wet weight) in marine species measured in this study with those in different locations in the world

Location	Concentration	Marine species	Reference
Korean coast	0.001-0.02	Fish (Anchovy, Flounder, Mackerel, Herring and Rock fish)	This study
Korean coast	0.005-0.22	Crustacean (Hair crab, Snow crab, Red snow crab and Blue crab)	This study
Korean coast	0.009-0.20	Bivalve (Mussel, Oyster and Clam)	This study
Korean coast	0.04-0.45	Bivalve (Mussel and Oyster)	Kim et al.[25]
Tyrrenian Sea, Italy	0.03-0.05	Clam	Binelli and Provini[29]
West coast, Sri Lanka	0.044	Clam	Guruge and Tanabe[30]
West coast, Sri Lanka	0.033-0.056	Crab (Blue crab and Mud crab)	Guruge and Tanabe[30]
Tokyo Bay, Japan	0.64-4.4	Fish (Ayu, Blue gill, Hass, Sea bass, Mullet, Croaker and Konoshiro)	Guruge et al.[31]
West coast, Sri Lanka	0.012-0.57	Fish (Mullet, Cat fish, Silver batfish, Rabbit fish and Flounder)	Guruge and Tanabe[30]
Koh Kong, Cambodia	0.01-0.18	Fish (Croaker, Mullet, Mackerel and Snapper)	Monirith et al.[32]
South Sinai, Egypt	ND*-20.3	Fish (Bouri, Denis, Moza and Mousa)	Nemr and Abd-Allah[33]
White Sea, Russia	0.06-2.82	Fish (Navaga, bullrout, Cod, Herring and Smelt)	Muir et al.[34]
Black Sea, Turkey	0.32-2.4	Fish (Anchovy, Whiting, Mullet, Mackerel and Goby)	Tanabe et al.[35]
Mediterranean Sea	0.6-10	Fish (Mullet and Sea bass)	Pastor et al.[36]
South coast, Norway	0.04-0.12	Fish (Cod, Dab, Plaice and Lemon sole)	Green and Knutzen[37]

ND* : not detected.

Table 3. Estimated daily dietary intake of HCB by marine organism consumption in Korea

	Average intake* (g/day)	Daily dietary intake	
		HCB (pg/kg body weight/day)	HCB_{-TEQ} (pg-TEQ/kg body weight/day)
Fish	30.5	2.61	0.26×10^{-3}
Crustacean	3.3	5.21	0.52×10^{-3}
Bivalve	4.4	4.58	0.46×10^{-3}
Gastropod	0.5	0.05	0.005×10^{-3}
Cephalopod	7.8	0.30	0.03×10^{-3}
Total	46.5	12.7	1.27×10^{-3}

*Data were taken from Ministry of Health and Welfare (MOHW)[24].

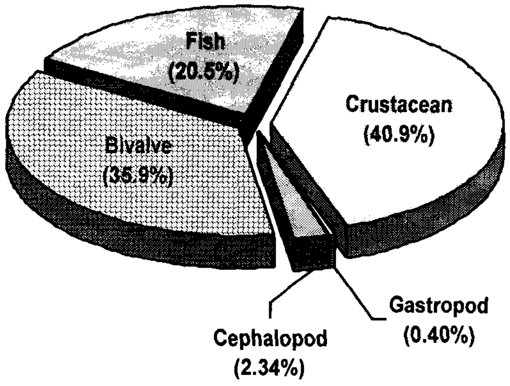

Fig. 3. Relative contribution to the estimated HCB dietary intake (pg/kg body weight/day) through marine organism ingestion in Korea.

sified as a dioxin-like compound, considering the binding to Ah-receptor, the dioxin-like effects, and the bioaccumulation in higher trophic levels. It was estimated that HCB is about 10,000 times less potent than 2,3,7,8-tetrachloro dibenzo-*p*-dioxin (2,3,7,8-TCDD). Based on above toxicological result, the dietary intake of HCB_{-TEQ} by marine organisms consumption in Korea was estimated to be 1.27×10^{-3} pg-TEQ/kg body weight/day. The dietary intake of HCB_{-TEQ} in fish was 0.26×10^{-3} pg-TEQ/kg body weight/day. Crustacean's dietary intake for HCB_{-TEQ} was 0.52×10^{-3} pg-TEQ/kg body weight/day. In bivalve species, it was 0.46×10^{-3} pg-TEQ/kg body weight/day. Gastropod and cephalopod species showed relatively low dietary intake of HCB_{-TEQ} in this study.

Recently, the World Health Organization (WHO) expert committee recommended a tolerable daily intake

(TDI) of 1-4 pg-TEQ/kg body weight/day[39]. In addition, a TDI for human exposure in the Korean population was proposed as 4 pg TEQ/kg body weight/day by the Korea Food and Drug Administration (KFDA). Most recently, the UK committee on toxicity of chemicals in food, consumer products and the environment has recommended a TDI of 2 pg TEQ/kg body weight/day[40]. In this study, the estimated dietary intake of $HCB_{\text{-TEQ}}$ (1.27×10^{-3} pg-TEQ/kg body weight/day) by marine organism consumption did not exceed the TDIs proposed by the KFDA, the WHO, and the UK toxicity committee. Therefore, the dietary intake of HCB by the consumption of Korean marine organisms was relatively safe for human health risk.

3.3. Human health risk assessment

In general, there are two methods of human health risk assessment from ingesting toxic organic contaminants. One is carcinogenic risk and the other is non-carcinogenic risk. HCB can be considered as a contaminants with cancer and non-cancer effects. The potential health risks of ingesting marine organisms contaminated with HCB were evaluated for the Korean adult population using the risk assessment method of the US EPA[14]. For carcinogenic effects, the risk is expressed as a cancer risk. The cancer risk below 1×10^{-6} means the adverse effects are negligible. The equation for estimating exposure of carcinogenic pollutants by marine organisms consumption is as follows:

$$CancerRisk = \frac{IFR \times C \times ED \times EF \times CSFo}{BW \times AT}$$

where IFR is the food ingestion rate (g/day); C is the concentration of chemicals in food (mg/g); ED is the exposure duration (adults=30 years); EF is the exposure frequency (350 days/year); CSFo is the oral cancer slope factor[41] (1.6 $(\text{mg/kg/day})^{-1}$, the data for individual chemicals were obtained from integrated risk information system (IRIS) reported by US EPA (2004))[41]; BW is the human body weight (adults=60 kg); AT is the average time for carcinogens (365 day/year×number of exposure years, assuming 70 years). From this equation, a cancer risk by marine organism consumption was 8.38×10^{-9} and the value is below 1×10^{-6} of cancer risk guideline. The result indicates that the level of exposure is not likely to cause any adverse effect in the Korean human population during their lifetime.

For non-carcinogenic effects, the risk is expressed as a target hazard quotient (THQ). If the THQ exceeds unity (1), an adverse effect might occur for a lifetime in human population. The equation for estimating exposure of non-carcinogenic pollutants by marine organisms consumption is as follows:

$$THQ = \frac{EF \times ED \times IFR \times C}{RfDo \times BW \times AT}$$

where RfDo is the oral reference dose (8×10^{-4} mg/kg/day)[41]. From this equation, a cancer risk was 6.54×10^{-6} and the value is below 1. The result indicates that the level of exposure is not likely to cause any adverse effect in the Korean human population during their lifetime.

Judging from this investigation, the HCB levels in marine organisms observed in the present study would not seem to cause cancer or reproductive disorders. However, HCB has been pointed out to act as a dioxin-like compound and the effects of low-dose exposure remain clear. Therefore, more intensive studies on HCB intakes by consumption will be necessary, in particular, for heavy fish consumers and their infants and fetuses. In addition, an adequate guideline on dietary intake of marine organisms should be established in Korea to protect human health from a risk by marine organism consumption.

4. Conclusions

Hexachlorobenzene (HCB) levels in various marine organisms (35 species) caught from the Korean coasts were analyzed. HCB was present in all kinds of marine organisms. HCB residues in all marine organisms ranged from 0.51 to 222 pg/g wet weight. Daily dietary intake of HCB by the consumption of marine organisms was estimated to be 13.4 pg/kg body weight/day. The relative contribution of each taxonomic group to the total dietary intake of HCB was in the order of crustaceans, bivalves, fish, cephalopods, and gastropods. Lifetime cancer risk and target hazard quotient (THQ) associated with marine organism consumption in Korean adult population were below risk guideline. This result suggests indicate that HCB ingestion through marine organisms seems to be safe for human health with negligible risk. The current residues of HCB in various marine organisms are not potential threats to public health in Korea.

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