

Immunological Impact of Metals in Harbor Seals (*Phoca vitulina*) of the North Sea

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Environmental pollutants may affect the immune system of marine mammals in many areas of the industrialized world. This study provides the first evidence for metal-induced hypersensitivity in harbor seals and demonstrates a relationship between this immunopathy and the level of metals in blood. The concentrations of 20 essential and nonessential elements were analyzed in the blood of 13 harbor seals from the North Sea. In addition, their T-lymphocyte response to metals in terms of hypersensitivity was investigated using a lymphocyte transformation test (LTT) according to the MELISA (memory lymphocyte immunostimulation assay) modification. The results showed metal hypersensitivities in 7 of 11 seals investigated in MELISA (data from two seals could not be assessed), reflecting a positive or possible positive reaction in 13 of 154 total single tests. Four animals responded to one metal and three animals to multiple metals. The sensitizing metals were molybdenum (Mo), titanium (Ti), nickel (Ni), chromium (Cr), aluminum (Al), lead (Pb), and tin (Sn). Furthermore, the seals with a Ni-, Al-, and Cr-sensibilization showed the highest concentrations of these metals in blood. In 8 of the 13 positive cases, elevated blood metal concentrations correlated with the hypersensitivity reaction. Summarizing, we demonstrate in this first pilot study the potential immunological impact of metals in seals, a topic rarely investigated previously. Our results show the value of a combined biological and effect-monitoring tool to investigate pollution-induced immunopathies in live animals.

Introduction

Metals are commonly found in the environment, and marine mammals have historically been exposed to them. Their

presence may be due to natural occurrence or from anthropogenic sources, both of which can result in toxic and immunotoxic effects on health. As top predators, marine mammals are exposed to metals predominately through their consumption of contaminated fish.

Numerous studies show that accumulation of metals occurs in different marine mammal species in the North and Baltic Seas (1–3). Most of these studies investigated metal concentrations in liver, kidney, or muscle, i.e., tissues available only through post-mortem examination. In living animals the choice of samples is limited and restricted for the most part to blood and hair. However, because of sampling difficulty, only few studies report values for metals in blood of marine mammals (4–6). Therefore, in the biomonitoring part of this study, the concentrations of 20 essential and nonessential elements (aluminum, arsenic, beryllium, cadmium, chromium, cobalt, copper, gold, iron, lead, manganese, molybdenum, nickel, palladium, platinum, selenium, silver, tin, titanium, zinc) were analyzed for the first time in the whole blood of harbor seals from the North Sea. Such results reflect recent exposure to metals and show the actual body burden.

Pollution with metals may affect the immunocompetence of free-ranging populations of marine mammals in many areas of the industrialized world. An imbalance of the immune system caused by pollutants has been suggested to play a role in the incidence of infectious diseases in marine mammals (7–10). Immune parameters such as natural killer cell activity, phagocytosis, and transformation of lymphocytes have been investigated for different species and provided evidence for the immunosuppression function of metal pollutants (11–14). In addition to immunosuppression, immunotoxic pollutants may induce dysregulation of the immune response, leading to hypersensitivity and autoimmunity. In human medicine it is known that hypersensitivity and autoimmune reactions can participate in the etiology of serious systemic diseases, but there is a lack of investigations in marine mammals. Even though the metal input in the marine system appears to be decreasing in the last years, low-level metal concentrations can modulate the immune system. The chronic intake of metal pollutants renders marine mammals candidates to develop hypersensitivity reactions.

A lymphocyte transformation assay (LTT) can be used for the demonstration of specific sensitizations toward infectious antigens (15) but also for the diagnosis of allergies to drugs, metals, toxins, or other chemicals (16–19). The method is based on the fact that lymphocytes, which have been sensitized by a certain antigen (“memory cells”), transform into blasts and proliferate when they are reexposed to this antigen. In the effect-monitoring part of this study, we used an LTT to determine metal hypersensitivity according to the MELISA (memory lymphocyte immunostimulation assay) modification (16, 17, 20, 21) as an effect-monitoring tool. Here we report metal-specific delayed-type hypersensitivity in harbor seals from the Danish and German Wadden Sea. This study provides evidence for metal-specific hypersensitivity in free-living animals and suggests a relationship between blood levels of metals and an immunological dysfunction in marine mammals.

The combination of the measurement of the actual metal body burden and the determination of metal hypersensitivities caused by recent or former contact was investigated for the use as a tool for monitoring marine metal pollution and its potential hazardous impact on the immune system of marine mammals.

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FIGURE 1. The seals were sampled at the Danish and German Wadden Seas.

TABLE 1. Details of Seals Analyzed in This Study

seal no.	date of blood sampling ^a	location	sex ^b	age ^b	length (cm)	weight (kg)	MELISA	
							no. of metal-specific tests	metal reactivities with SI > 2
01	09.04.2003	Lorenzenplate	m	sy	161	71	13	Ti (2.51)
02	09.04.2003	Lorenzenplate	m	sy	144	68	12	Ti (2.15)
03	09.04.2003	Lorenzenplate	m	sy	148	63	12	Pb (2.31) Mo (2.08)
04	09.04.2003	Lorenzenplate	f	py	140	47	17	Sn (2.36) Ti (2.97)
05	17.09.2003	Lorenzenplate	m	py	160	54	not assessed	
06	17.09.2003	Lorenzenplate	m	sy	168	69	not assessed	
07	02.12.2003	Römö	m	sy	166	—	14	—
08	02.12.2003	Römö	f	sy	165	—	14	—
09	02.12.2003	Römö	f	ty	100	23	8	—
10	13.04.2004	Römö	m	sy	172	91	9	Cr (2.37) Mo (2.72)
11	13.04.2004	Römö	m	sy	170	81	15	Ni (3.77) —
12	13.04.2004	Römö	m	sy	170	82	20	Al (3.89) Cr (2.26)
13	13.04.2004	Römö	f	sy	170	106	20	Ni (3.26) Mo (2.17)

^a Format for date: day.month.year. ^b Abbreviations: m = male, f = female, sy = several years, py = previous year, ty = this year.

Experimental Section

Animals. During 2003 and 2004, four seal catch events were carried out at the Danish and German Wadden Seas and blood samples taken for the planned analyses (Figure 1). The age was estimated based on length and weight and expressed as animals of this year (ty), of the previous year (py), and of several years (sy). Details of seals analyzed and the investigations performed are shown in Table 1. The routine medical checks, blood counts, distemper antibodies, and bacterial investigations showed no evidence of disease in these animals at the time of examination.

Lymphocyte Transformation Assay (MELISA). Blood was collected into monovettes with CPDA solution (citrate-phosphate-dextrose-adenin) (Sarstedt AG & Co., Nümbrecht, Germany) and stored at room temperature until

further processed (not longer than 15 h). The blood was diluted 1:1 with phosphate-buffered saline (PBS). Lymphocytes were separated on a Ficoll-Histopaque gradient (Sigma-Aldrich Chemie GmbH, Deisenhofen, Germany) and washed twice in PBS. Cells were resuspended in 20% medium [RPMI-1640 containing Hepes (Life Technologies GmbH, Karlsruhe, Germany) supplemented with 6.25 mM L-glutamine (Biochrom AG Seromed, Berlin, Germany), 8 mg/L gentamycin (Sigma-Aldrich Chemie GmbH), and 20% serum (seal 1–9, fetal calf serum from Biochrom AG Seromed; seal 10–13, 1:1 fetal calf serum and pooled human AB serum from Sigma-Aldrich Chemie GmbH)] and incubated in tissue culture flasks for 30–45 min at 37 °C in 5% CO₂ for depletion of monocytes. The lymphocytes were counted and resuspended in 10% medium to a concentration of 1 × 10⁶ lymphocytes/mL. One

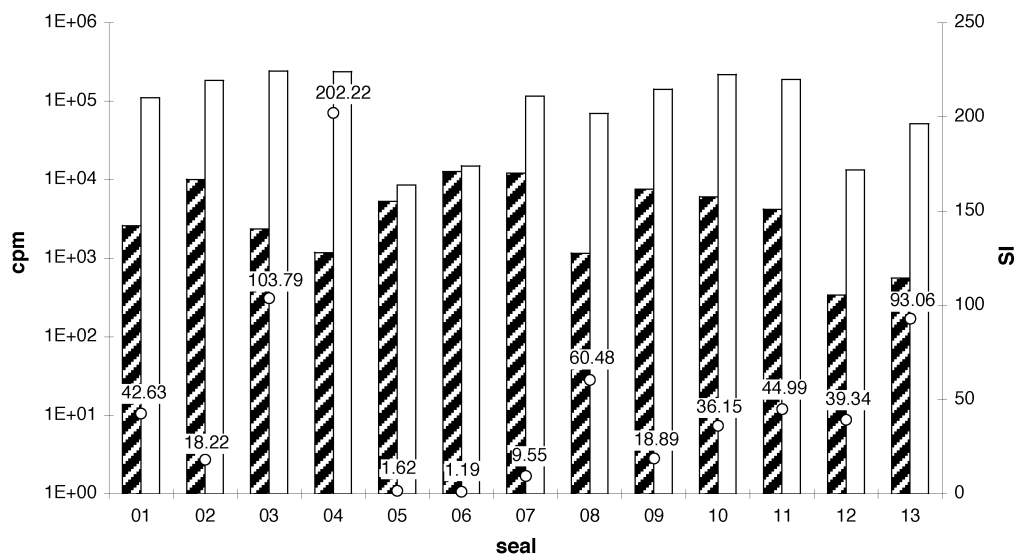


FIGURE 2. Mitogen-stimulated lymphocyte proliferation (positive controls) expressed as counts per minute (cpm, white column) and stimulation index (SI, points); nonstimulated lymphocyte proliferation (negative controls) is expressed as cpm (crosshatched column) in each individual seal (no. 01–13).

milliliter of cell suspension was pipetted into the wells of a 24-well plate (CM-Lab, Vordingborg, Denmark) precoated with metal salts. Depending on the number of lymphocytes obtained (variable among the seals), we tested the following metal salts: Ag, Al, Au, Be, Cd, Co, Cr, Cu, ethylmercury (EtHg), mercury chloride (HgCl), In, methylmercury (MeHg), Mo, Ni, Pb, Pd, phenylmercury (PhHg), Pt, Sn, and Ti. The metals were tested in three concentrations, I, II, and III. Level III is the highest, level II is the 1:1 dilution of level III, and level I the 1:1 dilution of level II. The concentrations of level III are given in $\mu\text{g}/\text{well}$: Ag (5), Al (40), Au (6.25), Be (50), Cd (6.25), Co (10), Cr (5), Cu (0.5), EtHg (0.5), HgCl (0.5), In (1), MeHg (0.5), Mo (25), Ni (5), Pb (25), Pd (6.25), PhHg (0.5), Pt (6), Sn (25), and Ti (50). One positive control with 2 $\mu\text{g}/\text{mL}$ poke weed mitogen (PWM, Sigma-Aldrich Chemie GmbH) was included in each test. Three negative control cultures without antigens provided information about the spontaneous proliferation of the lymphocytes. Where sufficient lymphocytes were available, replicate tests were performed to validate the intraassay variability. After 5 days of incubation at 37 °C in 5% CO₂, 600 μL of each cell suspension was transferred to a new plate and incubated for a further 4 h with 3 μCi radiolabeled methyl-³H-thymidine (Amersham Buchler GmbH & Co. KG, Braunschweig, Germany). The cells were then harvested onto filters, and the radioactivity was measured in a scintillation counter (1450 Microbeta Trilux Wallac Distribution GmbH, Freiburg, Germany). The incorporation of thymidine (cpm) in the stimulated well compared to negative control cultures defines the stimulation index (SI): $\text{SI} = \text{stimulated well (cpm)} / \text{mean value of three negative controls (cpm)}$. $\text{SI} \geq 3$ is regarded as a positive response, SI between 2 and 3 is interpreted as a possible sensibilization, and $\text{SI} < 2$ is considered negative. $\text{SI} < 2$ for the positive control indicates a functional disorder of the lymphocytes; these animals were not assessed.

In addition, to confirm the presence of lymphoblasts in positive wells and the lack of cytotoxicity in negative wells, 150 μL of cell suspension of the relevant wells was centrifuged (5 min, 1000 rpm) in a cytospin (Thermo Shandon, Pittsburgh, PA) onto microscope slides, stained with Diff-Quick solutions (Dade Behring Marburg GmbH, Marburg, Germany) according to the manufacturers protocol, and analyzed microscopically.

Determination of Elements in Blood. Blood samples were collected in lithium heparin monovettes for metal analysis

(Sarstedt AG+Co) and stored at $-20\text{ }^\circ\text{C}$. Prior to multielement determination, a Berghoff Pressure Digestion System (Berghoff Laborprodukte GmbH, Eningen, Germany) was applied. The TeflonTFM-inserts were filled with 500 μL of whole blood combined with 1450 μL of sub-boiled nitric acid, and 50 μL of internal standard (Yttrium; Merck, Darmstadt, Germany). The mixture was heated for 3 h at 180 °C. Concentrations of 20 trace elements were determined with three different methods. Ag, Al, Be, Cd, Co, Cr, Mn, Mo, Ni, Pb, Pd, Pt, and Sn were analyzed using an inductively coupled plasma-mass spectrometer (ICP-MS) with a collision cell (Agilent 7500c ICP-MS, Agilent Technologies, Tokyo, Japan). Matrix effects and instrumental drift of the ICP-MS were corrected by using yttrium as an internal standard. For calculation, external calibration was made by diluted standard solutions (Merck). The standard mode was used for Al, Be, Pb, Pd, and Sn. For the other elements, better results were obtained with He as collision gas (flow rate 3.0 mL/min).

Measurements of As, Au, Cu, Fe, Se, and Zn, on the other hand, were performed by total-X-ray-fluorescence spectrometry (TXRF) (Atomika TXRF 8030 C, FEI Co., Oberschleissheim, Germany). Digested samples (20 μL) were pipetted onto the sample carrier and evaporated to dryness. The Mo K α excitation was selected for detecting the elements. Yttrium as internal standard was used to calculate the results.

The level of Ti was determined by a high-resolution sector field ICP-MS (ELEMENT, Thermo Finnigan MAT GmbH, Bremen, Germany). Since the Ca concentration in blood and serum is very high, the Ti determination via the most abundant isotope ⁴⁸Ti is not possible via high-resolution ICP-MS because of the isobaric interference by ⁴⁸Ca (0.2%). Additionally, ⁴⁶Ti is also excluded for determination due to the presence of ⁴⁶Ca (0.03%). Finally, overlap with the ⁵⁰V⁺ and ⁵⁰Cr⁺ ion signals made determination using the ⁵⁰Ti isotope impossible. Therefore, the level of Ti was determined via the isotopes ⁴⁷Ti and ⁴⁹Ti. Quantification was carried out using single standard addition as a calibration technique.

For internal quality control the reliability of the analytical procedures was checked with the human reference material Clin Check Whole Blood Control Level II Lot No. 932 (Recipe, Chemicals+Instruments, Munich, Germany).

Statistical Analysis. We calculated an index of an individual's total contamination by first ranking metal concentrations across all subjects, separately for each metal, and then averaging the ranks for each subject, separately.

TABLE 2. Lymphocyte Responses to Metals

metal	no. of animals tested	no. of animals with SI ^a > 2		metal	no. of animals tested	no. of animals with SI ^a > 2	
		SI = 2-3	SI > 3			SI = 2-3	SI > 3
Ag	3	0	0	In	2	0	0
Al	3	0	1	MeHg	11	0	0
Au	6	0	0	Mo	11	3	0
Be	7	0	0	Ni	11	0	2
Cd	11	0	0	Pb	11	1	0
Co	7	0	0	Pd	8	0	0
Cr	10	2	0	PhHg	6	0	0
Cu	6	0	0	Pt	3	0	0
EtHg	8	0	0	Sn	9	1	0
HgCl	11	0	0	Ti	10	3	0

^a SI = stimulation index.

We used the nonparametric Mann–Whitney *U*-test for comparing metal concentrations in blood between the two locations, Römö and Lorenzenplate. Due to small sample sizes and tied observations, we indicate exact *P*-values for the Mann–Whitney *U*-test (22, 23). All *P*-values are two-tailed. Since we also tested for a difference between the two locations considering a total of 20 metal concentrations, separately, we had to adjust *P*-values for multiple testing. We did this using Fisher's Omnibus test (24). This test

combines several *P*-values into a single χ^2 -value with degrees of freedom equaling twice the number of *P*-values.

We also applied Fisher's Omnibus test for combining *P*-values of Mann–Whitney *U*-tests comparing metal concentration between individuals showing a certain metal specific sensitization (SI ≥ 2) and individuals not showing such a sensitization. Statistical significance was designated as *P* ≤ 0.05 .

Results and Discussion

The influence of metals on marine mammal health is poorly understood. The possibility to use biomonitoring and effect-monitoring tools for its clarification is very important. The aim of the present study was to analyze the metal content in whole blood and the T-lymphocyte response to metals in terms of hypersensitivity. Age- and sex-dependent differences in these investigations were not considered due to the small number in each group.

Applicability of MELISA for seals. The cpm values for the negative and positive controls as well as the SI values for the positive controls are summarized in Figure 2. In 11 cases, the resulting SI for the positive controls was >2 . For two seals (no. 05 and 06) both cpm values were similar, so the SI was <2 . These animals were not further assessed. The strong mitogen-stimulated lymphocyte proliferation and the relatively low level of spontaneous proliferation of the lymphocytes confirm the applicability of this LTT for seals. Con-

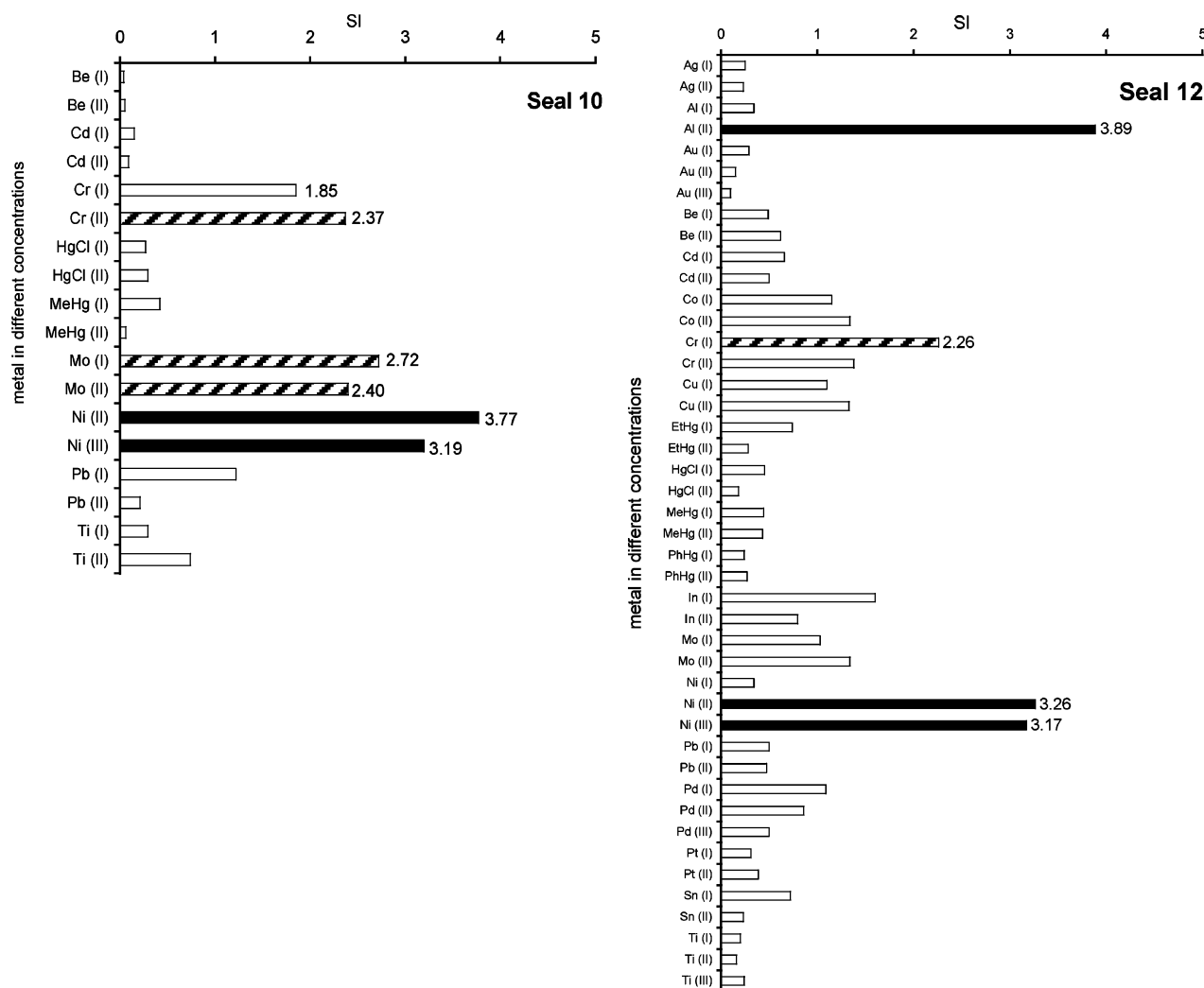


FIGURE 3. MELISA reactivity to selected metals in different concentrations of seals no. 10 and 12 (bars: filled = SI ≥ 3 , crosshatched = SI 2–3, white = SI ≤ 2).

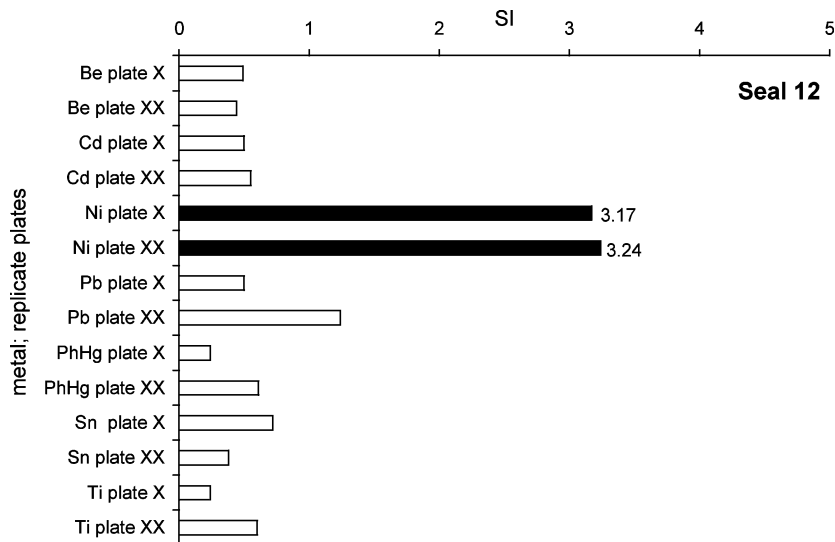


FIGURE 4. Intraassay variability: MELISA reactivity to seven metals, tested in duplicate (plate X and XX) from seal No. 12 (bars: filled = SI ≥ 2, white = SI < 2).

TABLE 3. Metal Concentrations (μg/L) in Blood from 13 Seals, Median Values, and Results of Mann–Whitney *U*-test (*U*- and *P*-value)

	Lorenzenplate						Römö							median	<i>U</i> -value	<i>P</i> -value
	01	02	03	04	05	06	07	08	09	10	11	12	13			
Ag	13.6	10.6	4.00	4.72	46.8	23.3	26.7	<1.80 ^a	<1.80 ^a	19.6	7.70	6.20	33.3	10.6	18	0.731
Al	40.0	125.9	0.60	<0.17 ^a	35.1	35.4	499	51.8	385	499	16.2	557	48.8	48.8	6	0.034
As	76.9	69.3	92.5	70.8	141	108	147	235	73.0	206	118	75.1	194	108	9	0.102
Au	74.0	86.0	138	121	86.0	92.0	112	105	81.0	97.0	101	89.0	86.0	92.0	20	0.945
Be	<0.08 ^a	<0.08 ^a	<0.08 ^a	<0.08 ^a	<0.08 ^a	0.22	<0.08 ^a	<0.08 ^a	<0.08 ^a	<0.08 ^a	<0.08 ^a	<0.08 ^a	<0.08 ^a	<0.08 ^a	17.5	0.628
Cd	<0.12 ^a	<0.12 ^a	<0.12 ^a	<0.12 ^a	0.32	0.16	0.20	3.10	0.30	0.16	0.12	0.16	0.15	0.16	11	0.181
Co	0.23	0.19	0.16	0.48	0.79	1.29	6.10	0.54	0.37	7.56	0.82	1.36	<0.02 ^a	0.54	12	0.234
Cr	20.6	18.4	17.0	19.4	14.7	8.76	19.3	10.3	31.8	38.6	7.42	50.2	31.5	19.3	13	0.294
Cu	812	792	850	780	723	732	986	774	850	765	954	838	907	812	9.5	0.101
Fe	664775	751997	693242	626973	620963	636412	860445	598812	725202	865252	713773	755544	789690	713773	8	0.074
Mn	141	151	118	94.5	61.5	81.8	105	93.7	140	79.0	90.4	88.2	75.2	93.7	15	0.446
Mo	2.40	1.52	1.52	2.80	1.46	4.84	2.47	10.8	1.46	4.68	1.27	4.80	7.34	2.47	15	0.445
Ni	4.80	2.00	2.00	4.52	0.40	0.54	2.14	13.6	5.00	17.9	2.10	23.0	<0.38 ^a	2.14	10	0.138
Pb	0.73	1.05	0.63	<0.02 ^a	2.00	1.28	0.05	0.07	0.06	0.09	0.05	0.07	0.13	0.09	7	0.052
Pd	0.28	0.20	0.16	<0.12 ^a	<0.12 ^a	<0.12 ^a	<0.12 ^a	2.86	6.06	1.26	0.60	0.22	1.14	0.22	5.5	0.022
Pt	0.11	0.09	0.10	<0.04 ^a	0.17	0.30	0.13	8.30	8.28	1.00	<0.04 ^a	<0.04 ^a	0.92	0.13	13	0.295
Se	826	1145	2261	834	1056	696	678	518	1069	1372	946	920	934	934	18	0.730
Sn	0.21	<0.06 ^a	<0.06 ^a	<0.06 ^a	<0.06 ^a	<0.06 ^a	<0.06 ^a	<0.06 ^a	<0.06 ^a	<0.06 ^a	<0.06 ^a	<0.06 ^a	<0.06 ^a	<0.06 ^a	17.5	0.628
Ti	2.09	1.34	1.58	2.02	1.68	0.58	9.20	1.10	1.13	1.72	10.8	3.32	3.49	1.72	12	0.234
Zn	3234	3399	3195	3082	2966	2745	3615	2730	3282	3494	3830	3661	3847	3282	7	0.052

^a Limit of detection.

canavalin A (ConA) and PWM as powerful mitogens for inducing proliferative responses of peripheral blood mononuclear cells and evaluating cell functionality have been applied to harbor seals by other research groups (25, 26). As expected from these studies, we found a strong PWM-induced lymphocyte proliferation in 11 of 13 cases.

Metal-Specific Hypersensitivity Reactions in Seals. From 13 animals investigated with MELISA, the results from 11 were valid and could be assessed. Seven animals reacted to at least one metal with SI higher than 2 (Table 1). Four seals were negative to all metals, while four seals had an SI ≥ 2 to one metal (single reactivity) and three seals to more than one metal (multiple reactivity). The frequency of sensitizing metals was Mo and Ti (3 seals), followed by Ni and Cr (2 seals), and, finally, Al, Pb, and Sn (one seal each) (Table 2). Of 154 single tests performed, 13 results showed a possible positive (*n* = 10) or positive (*n* = 3) reactivity (Table 2).

This study suggests that seals, like humans, can develop metal hypersensitivity when exposed to a contaminated environment, depending on individual immunity, duration of exposure to pollutants, as well as diet/nutrition. Metals

found here to induce hypersensitivity in seals are known allergens in humans. In a study of 550 patients, responses to Ni (69%), Ti (21%), Mo (13%), Pb (6%), and Sn (6%) were found (21). Ni was also the most frequent sensitizer in a large study of 3162 patients (17). Remarkable is the similar frequency of response between humans and seals, although most of the humans investigated showed clinical symptoms and the number of seals investigated was small: in a study of 930 patients, 25% reacted to one and 37% to more than one metal (17). Similarly, in a group of 550 patients, 32% showed a reactivity to one metal and 43% to more than one metal (21). While the influence of metal sensibility on marine mammal's health is not known, systemic reactions have been described for humans. Neuropathological changes in multisymptomatic patients correlated with T-lymphocyte pathology and metal-specific lymphocytes (27). An overrepresentation of Ni allergies among patients with chronic fatigue syndrome has been described (28).

Two seals, no. 10 and 12, showed metal-specific sensitizations with SI > 3 (Table 1, Figure 3). The reactivity to the tested metal concentrations varied, possibly reflecting

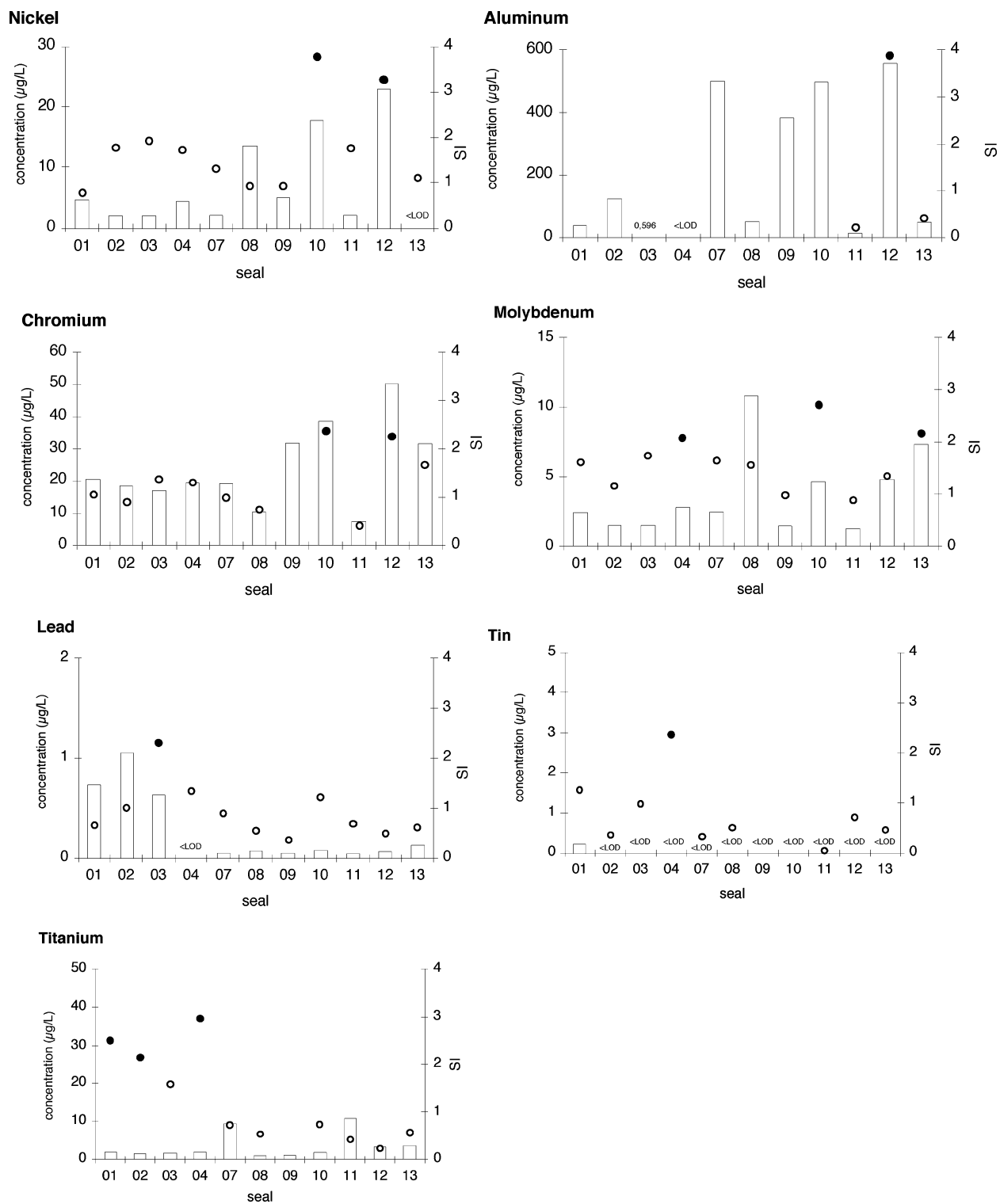


FIGURE 5. Correlation of metal concentration in whole blood ($\mu\text{g/L}$) (white bars, $<\text{LOD}$ = below the detection limit) and MELISA reactivity ($\text{SI} \geq 2$, black points; $\text{SI} < 2$, white points) in each individual seal (no. 01–13).

individual T-lymphocyte susceptibility (Figure 3). T-lymphocytes from both animals were sensitive to Ni and Cr and showed an additional individual metal-specific reaction; Mo for animal 10 and Al for animal 12.

In general, the individual immune status of marine mammals has been shown to depend on a complexity of factors such as age (14, 26), sex (12), duration of exposure to pollutants, as well as diet/nutrition (29–31).

MELISA Intraassay Variability. The intraassay variability could be tested against 7 metals in seal no. 12 (Figure 4). The

specific metal-induced lymphocyte proliferation was concordant negative for Be, Cd, PhHg, Pb, Sn, and Ti and concordant positive for Ni on two different plates. Positive results of a seal investigated over several months (interassay variability) were also concordant (manuscript in preparation). A similar high level of reproducibility was reported for humans in 250 (20) and, more recently, in 550 patients (21).

Determination of Elements in Blood. The values and medians of concentrations for each metal are shown in Table 2. Overall, the contamination of subjects from Römö was

higher than that of subjects from Lorenzenplate. In fact, indices of individuals' total contamination were on average higher in Römö (Mann–Whitney U -test: $U = 1$, $N_{\text{Lorenzenplate}} = 6$, $N_{\text{Römö}} = 7$, $P = 0.002$), and testing all metals separately also revealed an overall difference between the two locations (Fisher's Omnibus-test: $\chi^2 = 65$, $df = 40$, $P = 0.007$). Specifically, blood concentrations of Al and Pd were significantly different between the two locations ($P < 0.05$) while Fe, Zn, and Pb showed a definite trend ($0.05 < P < 0.1$) (Table 3).

When categorizing seals into sensitized ($SI \geq 2$) and not sensitized ($SI < 2$), we found a definite trend ($0.05 < P < 0.1$) for metal-sensitized seals to show higher blood concentrations of the corresponding metal (Fisher's Omnibus-test: tested metals Ni, Mo, Cr, Pb, Sn, Ti; $\chi^2 = 18.9$; $df = 12$, $P = 0.09$). For Ni and Cr this observation was statistically significant (Mann–Whitney U -test: Ni, $U = 0$, $N_{\text{sensitized}} = 2$, $N_{\text{nonsensitized}} = 9$, $P = 0.018$; Cr, $U = 0$, $N_{\text{sensitized}} = 2$, $N_{\text{nonsensitized}} = 8$, $P = 0.044$).

Altogether, 8 of 13 MELISA results with a $SI > 2$ coincided with elevated metal levels in blood (Figure 5).

Generally, high concentrations in blood indicate a recent metal intake, e.g., with food. Blood values reflect metal concentration in the circulating blood stream and define the actual body burden, dependent on the half-life of the metal under study. In humans, Ni increases in serum 4 h after Ni ingestion and decreases after 24 h (32). Similarly, during an intravenous infusion of Al^{3+} for 1 h, the plasma level of Al increased rapidly and decreased immediately following cessation of infusion (33). The half-life of Pb in blood is about 2–4 weeks (34).

However, the combination of a high metal concentration in blood with a metal-specific sensibilization strongly suggests chronic metal exposure. This can be caused predominately by chronic ingestion of contaminated food. The metal intake with food can vary with diet, feeding location, and feeding behavior (3, 35).

In addition to food intake, the presence of "endogenous" sources can induce a permanent release of metal into the blood stream. In humans it could be shown that the skeleton contains about 90% of the Pb body burden, which is continuously mobilized and presents a considerable "endogenous" Pb exposure (36). High Pb concentrations were also observed in bone and skin of Dall's porpoises and other marine mammals (37).

On the other hand, some of the relevant elements in this study do not appear to bioaccumulate significantly in mammalian tissues. Lower concentrations of Cr, Mo, Ni, and Pb in dolphin tissues compared with levels in their prey are described, possibly due to increased metal excretion by this species (38). Most Ni, Cr, and Pb levels are low in liver, muscle, kidney, and blubber in marine mammals in general (39) and in the marine mammals of the North and Baltic Seas in particular (3, 40, 41). Concerning Ti, immunological effects in humans have been described (42, 43), but corresponding investigations on marine mammals are extremely rarely. Some studies reported Ti in tissues of stranded dolphins and sperm whales, but most values were below the detection limit (44, 45).

Summarizing, the results reported here show the value of a combined biomonitoring and effect-monitoring tool to investigate the effects of metal pollution in live animals. Hypersensitivity reactions, which have an increasing importance in the industrialized world, are also important in the marine ecosystem. Although this study lacks a control group of seals living in an uncontaminated environment (this is difficult to find in the North Sea), the results show an immunological dysfunction induced by metals in seal lymphocytes isolated from blood of animals living in the Wadden Sea. Immunological dysfunctions suggest an imbalance

in the health status of an organism. Further studies with a larger number of animals and with animals from other locations should be performed to confirm and extend these findings.

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