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## DIOXIN AND HEAVY METALS CONTAMINATION OF REINDEER OFFAL FROM RUSSIAN FAR NORTH REGIONS

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### Abstract

Reindeer herding is vitally important agricultural sector in Russian Far North regions. Meat, liver, kidneys and other offal are highly consumed by indigenous people and go for export, therefore problem of reindeer products safety related to chemical contamination is of both scientific and practical interest. Here, we report levels of dioxins, dl-PCBs, cadmium and mercury in reindeer meat, liver and kidneys determined in 704 individual samples of meat, liver and kidneys of reindeers (*Rangifer tarandus tarandus* and *Rangifer tarandus sibiricus*) from 8 main reindeer-herding regions of Russia within the broad geographical range from western to eastern border of the country, including Kola Peninsula, Nenets Autonomy Orkrug and Yamalo-Nenets Autonomy Orkrugs, Taymir Peninsula, Kamchatka and Chukotka. Stable organic pollutants, including dioxins (polychlorinated dibenzo-para-dioxins and polychlorinated dibenzofurans) and dioxin-like polychlorinated biphenyls, were determined by high-resolution chromatography-mass spectrometry and expressed as WHO toxic equivalents (WHO-TEQ). Toxic elements were determined by mass spectrometry with inductively coupled plasma. Dioxin pollution has shown clear geographical distribution. It reaches the highest level at the Kola Peninsula and decreases to the East. Heavy metal pollution did not show any geographical trends. In particular, dioxins concentration in reindeer liver varied from  $42.2 \pm 10.6$  pg WHO-TEQ/g of fat in Kola Peninsula to  $1.2 \pm 0.6$  WHO-TEQ/g of fat in Chukotka. We have also investigated cadmium and mercury levels in different feed samples (lichen, mushrooms, compound feed and forage grain). Literature analysis showed a significant decrease of dioxin levels in reindeer liver over the last 15 years, while cadmium and mercury content in reindeer liver and kidneys has increased dramatically. Causes of clear geographical distribution of dioxin pollution and significant rise of heavy metal contamination need further research. It was shown that cadmium poses the highest health risk. Consumption of reindeer offal in six out of 8 investigated regions may lead to cadmium intake exceeding the tolerable intake level more than threefold. In the meantime, consumption of reindeer meat poses no health risk related to any of the investigated contaminants. Taking into account growing interest to reindeer liver processing for food production, it is usable to take into account our data on possible risk of longtime consumption of reindeer offal.

Keywords: *Rangifer tarandus* L., reindeer, dioxins, polychlorinated biphenyls, cadmium, mercury, persistent pollutants, Far North

Deer herding is an important agricultural industry of the Russian Federation. Total reindeer stock at the national territory reaches nearly 1.5 million of animals [1]. Deer meat is one of the main food products in core nations at the north of Russia and, thus, analysis of deer meat and sub-product contamination by dioxins and toxic elements is important [2-4]. It is believed that Northern ecosystems are apt to accumulation of sustainable contaminants since they have all necessary characteristics, including climate and food chain specificities. Contaminants reach the highest values in organisms being at the top of food

chain. Accumulation of xenobiotics depends on genus, feeding preferences, and metabolic activity of animals. As to reindeer, seasonal accumulation and mobilization of fat during winter plays an important role. Lipophilic compounds accumulated in fat tissue, in particular dioxins and dioxin-like polychlorinated biphenyls (dPCB), penetrate into other organs and tissues during fat mobilization [5-7]. Elective increase of dioxin accumulation occurs in liver of reindeer and sheep as compared to other productive animals (cows, pigs, poultry). Dioxins and dPCB are mainly penetrated into body in alimentary way (with feed and soils [8, 9]).

Increased dioxin and dPCB accumulation in reindeer and sheep liver are caused by specific biochemical properties (in particular, lower activity of detoxification enzymes, including cytochrome CYP1A); frequent change of pastures which increases probability of local point contaminated by dioxins; eating soil particles at pasturing [8]. Young reindeer show active penetration of dioxin and dPCB with mother's milk [10]. Increased dioxin accumulation is specific only for reindeer liver; their content in other organs and tissues, including muscles, kidneys, fat, blood, brain, and spleen is insignificant [11, 12].

During the entire year, vascular plants and lichen serve the main food for deer, where reindeer moss is the feeding base. Lichen makes over 50 % feed during winter. Accumulation of heavy metals in lichen at atmosphere contamination and active consumption of lichen by deer during winter are deemed to be the key factors defining the presence of heavy metals in organism [13]. Contamination of far North occurs mainly due to cross-border atmosphere and hydro-sphere transfer from other regions. For instance, only 13 % of mercuric contamination sources of Murmansk Region are located at the territory of the Kola Peninsula, 22 % — in other regions of Russia, and the other regions are Europe, China, America, Central Asia, etc. At the same time, industrial enterprises located at the territory of Far North, mainly mining ones (Severonikel of Kola Peninsula and Norilskiy Nikel of Krasnoyarsk Territory), also contribute to contamination by dioxin and toxic elements [2]). Polychlorinated biphenyls were actively produced in the past for different needs, for instance, for oil, paints, and lacquers [14]. During studies of Russian Far North within the International Program of Monitoring and Assessment of Arctic State it was concluded on presence of active unidentified dPCB sources at the territory of Russian Arctic, which were probably storage places of non-disposed PCB stocks [2].

Long-term effect of increased dioxin and dPCB concentrations negatively affects immune, nervous, and endocrine systems, sexual function and fetal development, and causes oncologic diseases [15]. Cadmium is first of all toxic for kidneys, may cause serious disorders in their function up to renal insufficiency. Accumulation of metal results in bone demineralization. Its effect is also due to growth of occurrence frequency of lung, endometrial, bladder, and breast cancer [16]. Mercury has toxic effect on developing nervous system, negatively affects the immune, blood, and sexual systems, liver and kidneys [17].

In present study we for the first time have identified content of toxic contaminants in reindeer meat and sub-products with reference to geographic regions and have assessed the risk at consumption of deer kidneys, liver, and meat. The highest dioxin contamination of reindeer liver was noted at Kola Peninsula, in furtherance its degree was decreasing when moving from West to East. We have not found similar dependence for cadmium and mercury.

Our purpose was assessment of the composition of dioxin, dioxin-like polychlorinated biphenyls, cadmium, and mercury in sub-products and muscular tissue of reindeers (*Rangifer tarandus tarandus* L. and *Rangifer tarandus sibiricus* Murray) from eight regions of Far North of Russia, studying of time-based con-

tamination dynamics by comparison with data from 15-year old literature sources, and estimation of xenobiotic penetration dosage to organism of the representatives of core northern nations.

*Techniques.* Reindeer tissue probes (704 individual samples of muscular tissue, liver, and kidneys) and feed (reindeer moss, mushrooms, combined feed, grain) were collected by inspectors of the Russian Service for Veterinary and Phytosanitary Surveillance within the scope of the state veterinary monitoring of food product and feed safety in years 2014–2016 in eight main deer breeding regions of the Russian Federation: Murmansk Region, Nenets Autonomous District (AD), Komi Republic, Yamal-Nenets Autonomous District, Taymyr Peninsula (Krasnoyarsk Territory), Sakha Republic (Yakutia), Kamchatka Territory and Chukotka Autonomous District. Tissues samples were frozen and delivered to the Russian State Center of Quality and Standardization of Medicinal Products for Animal Use and Feed (VGNKI, Moscow).

Dioxins (polychlorinated dibenzo-para-dioxins and poly-chlorinated dibenzofuranes) and dPCB were determined by approved methodology [18] with the use of a chromatography-mass-spectrometer Autospec Premier (Waters Corp., USA). Defrosted probes (50–100 g) were chopped and, after addition of internal standard solutions of isotope labeled dioxins and dPCB (Wellington Labs, Canada), wiped with sorbent Prep DE (Dionex, USA) until getting a homogenous mixtures. Afterwards, they were extracted under pressure by hexane-dichloride-methane solution 1:1 (vol.) (ASE 350, Dionex, USA). Extracts ere defatted ( $H_2SO_4$ /silica gel), purified on columns filed with 10 % activated carbon on celite, steamed and analyzed with the use of gas chromatograph with mass-spectrometric detection (GX-MK) in selected ion monitoring (SIM) mode at mass-spectrometric resolution no lower than 10 000 with capillary column VF-Xms (60 m×0.25  $\mu$ m, Agilent, USA). Total concentrations of dioxin congeners and dPCB were expressed in toxic equivalent units of World Health Organization (WHO TEU) and recalculated for fat content in the original sample. Fat content in probes was determined gravimetrically (State Standard GOST 23042-86 “Meat and Meat Products. Methods of Fat Determination”).

Toxic elements in samples were assessed according to the approved methodology [19]. Content of toxic elements (cadmium and mercury) was determined by mass-spectrometry method with inductively coupled plasma (ISP-MS) (mass-spectrometer Varian 820 MS, Varian, Australia). For measurements, samples were dissolved in nitric acid by microwave deterioration.

Overall dosage (OD) of dioxins and dPCB entering the organism was calculated by formula:  $OD(d) = AC(d) \times ACons(d)$ , where  $AC(d)$  is average concentration of total dioxins and dPCB in reindeer liver, kidneys or meat, pg WHO TEU/g of the total weight;  $ACons(d)$  is average consumption of liver, kidneys or meat, g/pax. per week. Due to lack of information on consumption of deer liver by inhabitants of Far North of Russia, liver consumption value by Sami in Northway of 64 g/pax. per week [13] was used in calculation, accounting for similar diet in deer breeding nations. Annual volume of consumed deer meat was taken from data by reindeer-breeding Chukchi (Chukotka Autonomous District) and comprised 72 kg/pax. per year [20]. Cadmium and mercury overall entering dosage  $OD(t)$ ,  $\mu$ g/pax. per week, was calculated by formula:  $OD(t) = AC(t) \times ACons(t)$ , where  $AC(t)$  is average content of cadmium or mercury in deer liver, kidneys or meat,  $\mu$ g/kg;  $ACons(t)$  is average consumption of deer liver, kidneys or meat, g/pax. per week. Since there is no information on consumption of deer kidneys by core nations of Russian Far North, the above indicated value for liver was used. Also, according to data collected for Eskimos of Northern Canada, core deer-breeding nations consume more deer kidneys than deer liver [21]. Obtained

results were compared with values of acceptable transferable weekly dosages of dioxin and dPCB, cadmium and mercury sum. For sum of dioxin and dPCB and cadmium, we have used values of 14 pg/kg and 2.5 µg/kg of human body weight per week as established by EU Scientific Committee on Food [15, 16]. For mercury, we used the WHO values of 4 µg/kg of human body weight per week [22]). WHO model average weight of 60 kg person was applied to recalculate values expressed per kilogram of body weight to the values per person [23].

Data on toxic element accumulation which were obtained for regions with sample number  $N > 10$  were involved in statistical processing. Average concentrations in several samples were subjected to dispersion analysis (ANOVA) for identification of statistical differences between regions. Values were statistically significant at  $P < 0.05$ . Mean values ( $\bar{X}$ ) and standard errors of the mean ( $\Delta$ ) are shown in tables below.

**Results.** Content of dioxins and dPCB in muscular tissue of reindeer does not exceed permissible values (see Table 1).

**1. Accumulation of dioxins and dioxin-like polychlorinated biphenyl (D and dPCB, pg WHO TEU/g of fat,  $\bar{X} \pm \Delta$ ) in reindeer (*Rangifer tarandus tarandus* L. and *R. tarandus sibiricus* Murray) in regions of Russian Far North (2014-2016)**

Region/number of samples	D	D + dPCB	D:dPCB, %
	Liver		
Murmansk Region/34	42.20±10.55	145.60±23.99	29:71
Nenets AD/155	23.90±5.98	85.20±13.95	28:72
Republic of Komi/10	20.71±5.18	—	—
Yamal-Nenets AD/79	14.01±3.50	—	—
Taymyr peninsula (Krasnoyarsk Territory)/22	5.06±2.43	18.60±5.34	29:71
Republic of Sakha (Yakutia)/5	1.10±0.53	—	—
Kamchatka Territory/5	3.50±1.68	—	—
Chukotka AD/10	1.20±0.58	—	—
Finland (3)/—	42	84.1	50:50
	Muscular Tissue		
Murmansk Region /24	1.30±0.62	—	—
Nenets AD/5	0.67±0.32	—	—
Taymyr peninsula (Krasnoyarsk Territory)/35	< 0.5	—	—

Note. AD — autonomous district. WHO TEU — toxic equivalent unit of World Health Organization. Permissible dioxin content in liver is 6, in muscular tissue — 3 pg WHO TEU/g of fat [24]. Dashes mean lack of data.

At the same time, number of hepatic dioxins in reindeer from Murmansk Region, Nenets Autonomous District, Republic of Komi, and Yamal-Nenets Autonomous District exceeded the acceptable limits. The highest hepatic dioxins and dPCB (7 times above the acceptable level) were found in deer from Kola Peninsula. dPCB comprised nearly 70 % of the total dioxin and PCB toxicity expressed in WHO TEU for all three studied regions. Dioxin contamination of deer meat was decreased at moving from West to East. Average dioxin content values at the territory of Northern Finland established by Finnish scientists in years 2006-2011 [5] well correlate with data obtained by us for Kola Peninsula. The dPCB contribution to total toxicity of persistent organic pollutants (POPs) for liver of reindeer at Finnish territory was significantly lower (50 %) than at territory of Kola Peninsula and other regions of Russia.

Concentration of cadmium and mercury in muscular tissue in reindeer is within the acceptable level (Table 2). However, values significantly grew in liver and kidneys. At that, no expressed decrease in content of heavy metals in organs and tissues is found when moving from west to east similar to the trend observed for dioxins. Cadmium level in reindeer moss (feeding base of reindeer) and mushrooms does not significantly exceed that in feed for bovine animals and pigs. Mercury level in reindeer moss was higher than in feed grain and insignificantly exceeded that in combined feed. That is, the obtained results disallow explanation of deer sub product contamination by heavy metals. Possibly, as in case of dioxins [8], reindeer differences from other species of productive animals (pasturing at

locally polluted territory, consumption of soil particles) play the key role. High concentration of cadmium and mercury in other types of deer's feed is also possible. Accumulation of mercury in mushrooms, for instance, was significantly higher than in other types of feed products (see Table 2).

**2. Content of cadmium and mercury in feed and organisms of reindeer (*Rangifer tarandus tarandus* L. and *Rangifer tarandus sibiricus* Murray) from different regions of Russian Far North (mg/kg,  $X \pm \Delta$ , years 2014–2016)**

Specimen	Region	Number of specimens	Cadmium	Mercury	
Liver	Murmansk Region	109	1.400±0.200	0.350±0.080	
	Nenets Autonomous District	155	0.260±0.040	0.170±0.070	
	Republic of Komi	10	0.620±0.090	0.260±0.060	
	Yamal-Nenets Autonomous District	78	0.720±0.110	0.180±0.080	
	Taymyr Peninsula (Krasnoyarsk Territory)	67	0.340±0.050	0.033±0.015	
	Republic of Sakha (Yakutia)	5	0.960±0.140	0.200±0.050	
	Kamchatka Territory	10	0.530±0.080	0.090±0.040	
	Chukotka Autonomous District	8	0.830±0.120	0.090±0.040	
	Kidneys	Murmansk Region	67	4.400±0.700	0.600±0.140
		Nenets Autonomous District	48	1.500±0.200	0.700±0.170
Republic of Komi		10	1.700±0.300	0.380±0.090	
Yamal-Nenets Autonomous District		49	4.300±0.600	0.580±0.140	
Taymyr Peninsula (Krasnoyarsk Territory)		10	0.810±0.120	0.060±0.026	
Republic of Sakha (Yakutia)		4	7.600±1.100	0.950±0.230	
Kamchatka Territory		10	2.500±0.400	0.410±0.100	
Chukotka Autonomous District		8	5.600±0.800	1.100±0.300	
Muscular tissue		Murmansk Region	6	0.012±0.004	0.110±0.050
		Nenets Autonomous District	5	< 0.005	0.020±0.009
	Yamal-Nenets Autonomous District	10	< 0.005	< 0.01	
	Taymyr Peninsula (Krasnoyarsk Territory)	35	< 0.005	< 0.01	
Reindeer moss	Murmansk Region	20	0.029±0.009	0.031±0.014	
Mushrooms (birch boletus)		3	0.042±0.013	0.084±0.037	
Reindeer moss	Republic of Sakha (Yakutia)	6	0.071±0.021	0.032±0.014	
Feed grain	Different regions	6	0.035±0.011	0.013±0.010	
Combined feed for cattle and pigs		10	0.055±0.017	0.027±0.012	

Note. AD — autonomous district. Permissible level (PL) of cadmium is 0.3 mg/kg for liver, 1.0 mg/kg for kidneys, and 0.05 mg/kg for muscular tissue; PL of mercury is 0.1 mg/kg for liver, 0.2 mg/kg for kidneys, and 0.03 mg/kg for muscular tissue [24].

The figure compares our results with data obtained in 2001 within the Program of Monitoring and Assessment of the Arctic State [2]. These data show is that dioxin content in organs and tissues of reindeer for the last 15 years had significantly decreased, whereas content of cadmium and mercury had significantly increased.

Calculation of the dosage of pollutants consumed by a person via reindeer meat, liver, and kidneys as compared to weekly permissible dosage are provided in tables 3, 4, and 5.

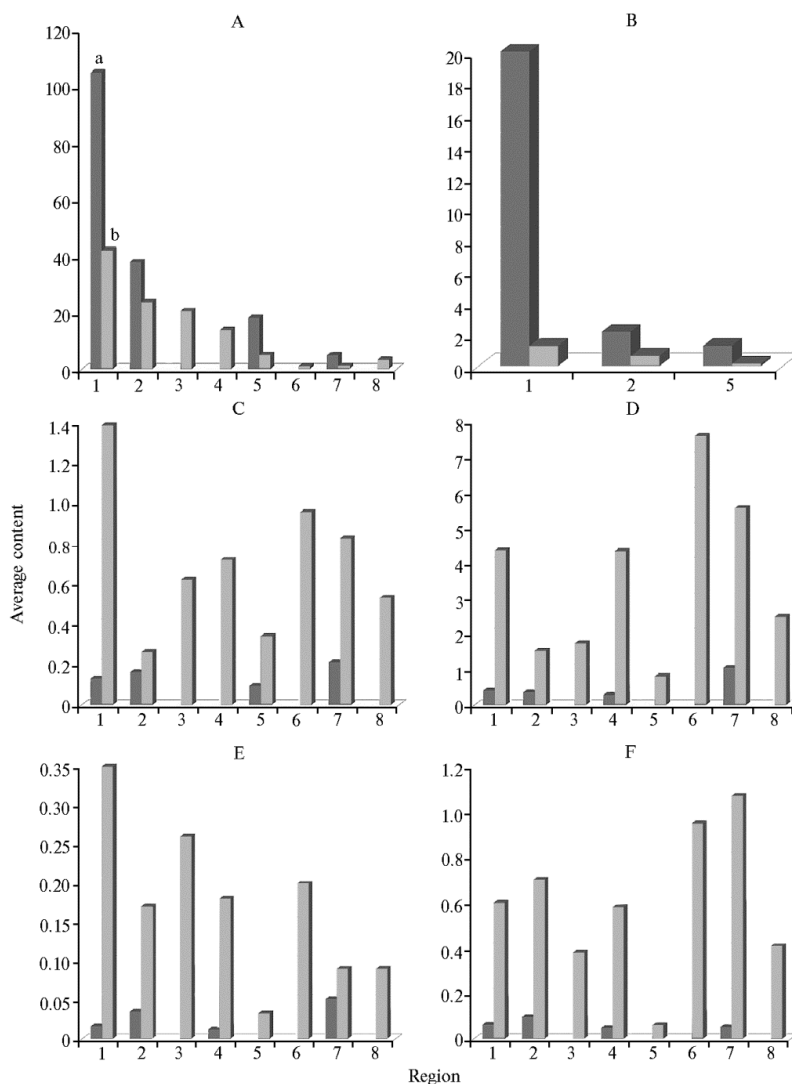
Consumption of reindeer liver resulted in entering of significantly higher dosages of dioxins and dPCB as compared to meat.

**3. Dosages of cadmium and mercury entering the body at consumption of reindeer (*Rangifer tarandus tarandus* L. and *Rangifer tarandus sibiricus* Murray) sub products and meat as compared to weekly permissible levels**

Region	Product, sub product	Dosage, mcg/(pax. · week)		% of PL	
		cadmium	mercury	cadmium	mercury
Murmansk Region, Nenets AD, Yamal-Nenets AD, Taymyr Peninsula	Meat	< 1	< 1	< 1	< 1
Murmansk Region, Nenets AD	Liver	88.96	22.40	59	9
Republic of Komi		16.64	10.88	11	5
Yamal-Nenets AD		39.68	16.64	26	7
		46.08	11.52	31	5

Taymyr Peninsula (Krasnoyarsk Territory)	21.76	2.11	15	1
Republic of Sakha (Yakutia)	61.44	12.80	41	5
Kamchatka Territory	33.92	5.76	23	2
Chukotka AD	53.12	5.76	35	2
Murmansk Region	278.4	38.40	186	16
Nenets AD	97.28	44.80	65	19
Republic of Komi	110.72	24.32	74	10
Yamal-Nenets AD	277.12	37.12	185	15
Taymyr Peninsula (Krasnoyarsk Territory)	51.84	3.84	35	2
Republic of Sakha (Yakutia)	486.40	60.80	324	25
Kamchatka Territory	159.36	26.24	106	11
Chukotka AD	355.84	68.48	237	29

Note. AD — autonomous district. Permissible level (PL) of cadmium is 150  $\mu\text{g}/(\text{pax} \cdot \text{week})$ , PL of mercury is 240  $\mu\text{g}/(\text{pax} \cdot \text{week})$ .



**Average dioxin content** (pg WHO TEU/g fat; A, B), **cadmium** (mg/kg; C, D) and **mercury** (mg/kg; E, F) in liver (A, C, E), muscular tissue (B) and kidneys (D, F) of reindeer (*Rangifer tarandus tarandus* L. and *Rangifer tarandus sibiricus* Murray) from different regions of Russian Far North: 1 — Murmansk Region, 2 — Nenets Autonomous District (AD), 3 — Republic of Komi, 4 — Yamal-Nenets Autonomous District, 5 — Taymyr Peninsula (Krasnoyarsk Territory), 6 — Republic of Sakha (Yakutia), 7 — Chukotka AD, 8 — Kamchatka Territory; a — data obtained in 2001 (the Program of Monitoring and Assessment of Arctic State — AMAP 2001), b — results obtained in years 2014-2016.

In the most dioxin-polluted Murmansk region dioxin and dPCB dosage affecting people via reindeer meat and liver is over half of permissible dose. For mercury, the most load results from reindeer kidneys, and average does at consumption of liver and kidneys does not exceed one third of PL. Situation with cadmium is extremely unfavorable since at consumption of only kidneys PL is exceeded in majority of regions. Total load at consumption of reindeer liver and kidneys exceeds PL in six of eight studied regions, except for Taymyr and Nenets AD, and reaches the highest values in Republic of Sakha (Yakutia) (is more than 3.5 times higher than the PL). Dosages of cadmium and mercury entering human body with reindeer meat in all four studied regions are no more than 1 % of a daily permissible dose.

**4. Total dosage of dioxin and dioxin-like polychlorinated biphenyl (D and dPCB, pg WHO TEU/g of total weigh) entering human body at consumption of reindeer (*Rangifer tarandus tarandus* L. and *Rangifer tarandus sibiricus* Murray) meat and liver as compared to weekly permissible level (PL)**

Region	Product, sub product	Fat, %	D + dPCB	Load on body, pg/(pax. · week)	% of TD
Murmansk Region	Meat	~ 5 (18)	0.0650	98	12
Nenets AD			0.0335	50	6
Taymyr Peninsula (Krasnoyarsk Territory)			0.0085	13	2
Murmansk Region	Liver	4.5	6.55	419	50
Nenets AD		6.0	5.11	327	39
Taymyr Peninsula (Krasnoyarsk Territory)		5.0	0.93	60	7

Note. AD — autonomous district, WHO TEU — toxic equivalents of World Health Organization. TD — 840 pg WHO TEU/(pax. · week),

**5. Total percentage of pollutants entering human body at consumption of reindeer (*Rangifer tarandus tarandus* L. and *Rangifer tarandus sibiricus* Murray) sub products and meat as compared to permissible level (PL)**

Region	% of PL		
	D + dPCB (liver + meat)	cadmium (liver + kidneys)	mercury (liver + kidneys)
Murmansk Region	62	245	25
Nenets AD	45	76	24
Republic of Komi		100	17
Yamal-Nenets AD		216	20
Taymyr Peninsula (Krasnoyarsk Territory)	9	50	3
Republic of Sakha (Yakutia)		365	30
Kamchatka Territory		129	13
Chukotka AD		272	31

Note. AD — autonomous district, D and dPCB — dioxins and dioxin-like polychlorinated biphenyl. Dashes mean lack of data.

It should also be noted that indigenous peoples of Russian Far North is subjected to significant effect of dioxins, dPCB and toxin elements and from other sources, besides deer meat, including food products, in particular marine mammals and fish [26].

Recently, high nutritional value of reindeer meat and liver is shown [25]. Also, nutritional value of reindeer liver is significantly higher than that of beef [27]. In using reindeer liver for cooking, it is reasonable to account for its contamination by persistent organic pollutants and toxic elements.

Therefore, dioxin contamination of reindeer liver in Russian Far North has clear geographical distribution, the most contaminated region is Kola Peninsula, in furtherance dioxin content is decreased when moving from west to east. Contamination of reindeer sub products by cadmium and mercury does not display similar regularity. For the last 15 years, content of dioxins in reindeer liver had significantly decreased, with the increase of cadmium and mercury level in liver and kidneys. Consumption of reindeer meat from the studied regions does not bear significant risk for health. But regular and long-term eating of reindeer

liver and kidneys could be risky because of more than 3-fold exceeded permissible doses.

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