

*Originalni članci/  
Original articles*

INFLUENCE OF DEKABROMINATED  
DIPHENYL ETHER ON CADMIUM AS  
OXIDATIVE STRESS INDUCER IN KIDNEY  
– SUBACUTE ORAL STUDY IN RATS

UTICAJ DEKABROMOVANOG DIFENIL  
ETRA NA TOKSIČNOST KADMIJUMA  
POSREDSTVOM OKSIDATIVNOG STRESA –  
SUBAKUTNA ORALNA STUDIJA NA  
PACOVIMA

**Correspondence to:**

**Marijana Ćurčić**

University of Belgrade – Faculty of  
Pharmacy, Department of Toxicology  
„Akademik Danilo Soldatović“,  
Vojvode Stepe 450,  
11221 Belgrade, Serbia;  
phone: +381 11 3951248;  
e-mail: marijana.curcic@pharmacy.bg.ac.rs

Marijana Ćurčić<sup>1</sup>, Marko Antunović<sup>2</sup>,  
Maja Džoković<sup>3</sup>, Saša Janković<sup>4</sup>,  
Vesna Milovanović<sup>5</sup>, Snežana Đorđević<sup>2</sup>,  
Vesna Kilibarda<sup>2</sup>, Slavica Vučinić<sup>2</sup>,  
Biljana Antonijević<sup>1</sup>

<sup>1</sup> University of Belgrade – Faculty of Pharmacy, Department of  
Toxicology „Akademik Danilo Soldatović“, Belgrade

<sup>2</sup> National Poison Control Center, Military Medical Academy, Belgrade

<sup>3</sup> Consultant in analytical biochemistry, Belgrade

<sup>4</sup> Institute of Meat Hygiene and Technology, Belgrade

<sup>5</sup> University Childrens Hospital Tiršova, Belgrade

*Key words*

cadmium, decabrominated diphenyl ether  
(BDE-209), oxidative stress

*Ključne reči*

kadmijum, dekabromovani difenil etar  
(BDE-209), oksidativni stres

*Abstract*

It is known that kidneys are target tissue for Cd toxicity but there is not enough literature data on kidney effects of BDE-209. Moreover, Cd as toxic metal and BDE-209 as organic halogenated pollutant can share similar mechanism of action like, oxidative stress which is already assumed. However, how will Cd act as prooxidant in presence of BDE-209 is still not investigated, therefore, the objective of this study was to assess this BDE-209 influence. Results of this study indicated slight decrease in MDA in rat's kidney homogenates after subacute exposure to Cd and/or BDE-209, while SOD activity was increased and content of –SH groups decreased. Based on dose-response assessment, BDE-209 did not influence Cd toxicity mediated by chosen oxidative stress parameters, namely the derived CEDL<sup>5</sup> values were the same for single Cd and for Cd given in combination with the BDE-209.

In conclusion, dynamic of oxidative process has influence on the parameters levels. Co-exposure with polybrominated organic pollutant did not influence Cd effects on oxidative stress parameters. Having in mind that this is pilot study, performed with only one dose of BDE-209, further investigations are necessary, including changes in dose ranges, duration of exposure etc. Moreover, results contributes to the issue of mixture toxicology even BDE-209 did not influenced oxidative stress as mechanism of Cd toxicity.

## INTRODUCTION

Cadmium (Cd) is widely distributed in the environment, either naturally or as a result of human activities, while decabrominated diphenyl ether (BDE-209) is mainly released from industrial processes or products containing this flame retardant<sup>1-7</sup>. Humans are exposed to a of Cd and BDE-209 mainly by inhalation or ingestion, which is confirmed by their presence in human tissues<sup>1-2,6-8</sup> which can result in toxic effects that are difficult to be identified based on information on single substances. It is known that kidneys are target tissue for Cd toxicity but there is not enough literature data on kidney effects of BDE-209. Moreover, Cd as toxic metal and BDE-209 as organic halogenated pollutant can share similar mechanism of action like, oxidative stress which is already assumed<sup>9-10</sup>. However, how will Cd act as prooxidant in presence of BDE-209 is still not investigated, therefore, the objective of this study was to assess this BDE-209 influence.

## MATERIALS AND METHODS

Male *Wistar* rats (weighing 220g) were kept in cages, under controlled conditions, at the air humidity of 40-60%, temperature of 20-24°C and under dark:light cycles 12h:12h. Food and water were available *ad libitum*. Study was done in accordance with the Law on animal welfare (Off. Gazette RS. No. 41/09) and decision of Ethical Committee of Military Medical Academy No. 9667-1/2011 - Ethical Committee, Military Medical Academy, Belgrade, Serbia.

Animals (6 to 8 per group) were given by gavage, single oral dose of Cd, BDE-209 or their three combinations during 28 days. Control groups were treated with water and dimethyl sulfoxide (DMSO). Three groups of rats were treated with 2.5, 7.5 or 15 mg Cd/kg/day. These doses were chosen on the bases of literature data exposure<sup>2,11-13</sup>. One group of rats was treated with 1000 mg BDE-209/kg/bw/day<sup>1,14</sup>. Furthermore, three groups of animals were treated with different doses of Cd in combination with dose of BDE-209. Purity of used chemicals was: BDE-209 (98%) (Sigma-Aldrich, St. Louis, MA, USA), CdCl<sub>2</sub> (CdCl<sub>2</sub>·xH<sub>2</sub>O, 99.99%) (Merck, Darmstadt, Germany) and (DMSO, 99.9%) (Sigma-Aldrich, St. Louis, MA, USA).

During the subacute exposure, water and food intake were recorded weekly, while body weight, clinical signs of toxicity and behaviour were recorded daily.

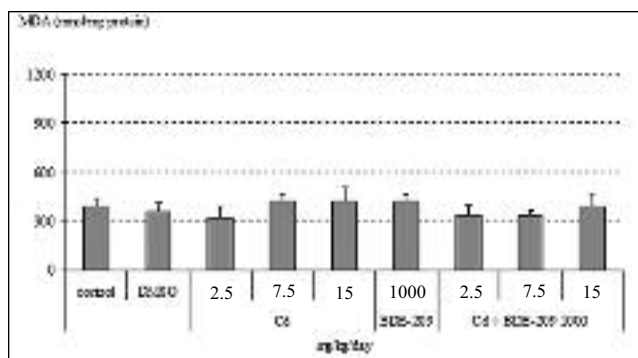
Kidneys were taken after the sacrificing the animals at the end of experiment, and part of kidney tissue homogenised for oxidative stress parameters analysis like we described in Curcic et al. (2015)<sup>15</sup>. Oxidative stress determination in kidney homogenates was based on: malondialdehyde (MDA) concentration, superoxide dismutase (SOD) activity, -SH groups content and morphological changes in the kidneys. The level of MDA in liver homogenates was based on its reaction with thiobarbituric acid under acidic condition for 15 min at 95 °C. In this reaction light yellow to pink colour complexes were formed depending on the concentration of MDA in tissue homogenates. Intensity of colour was measured at 523 nm and 600 nm. Activity of SOD, EC 1.15.1.1. in homogenates was measured using the method of Misra and Fridovich (1972)<sup>16</sup>, The total content

of -SH groups in liver homogenates was determined by the Ellman's method (1959)<sup>17</sup>.

Statistically significant differences among data ( $p < 0.05$ ) were tested using analysis of variance (ANOVA) followed by post hoc Tukey test (Statistica 7.0). Effect assessment i.e. dose dependent influence on measured parameters was done by PROAST software (Bilthoven, Netherland). Calculated Benchmark dose (CED in software) and its lower confidence limit (CEDL<sup>5</sup>) of 5% for the impact of 5% on parameter implied dose dependent manner, but only when ratio between CED and CEDL<sup>5</sup> is lower than 10<sup>18</sup>.

## RESULTS AND DISCUSSION

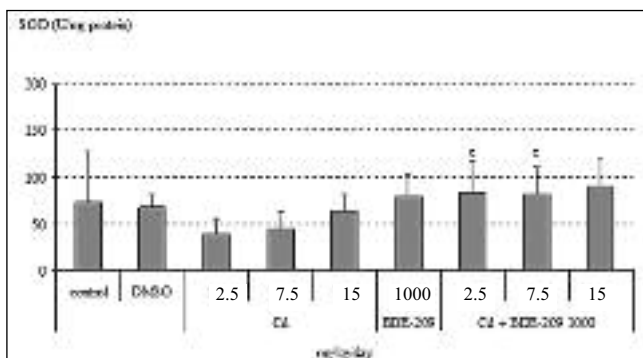
Results on MDA concentration imply that BDE-209 in dose of 1000 mg/kg/day did not have impact on this parameter. Calculated two CEDL<sup>5</sup> of 1.008 mg Cd/kg/day for Cd given alone or in combination with BDE-209 are the same and confirm this statement (Table 1). Even contradictory, the role of oxidative stress in Cd toxicity is evident<sup>19-20</sup>. First observations on this issue came from early 80ties and are related with Cd impact on normal mitochondrial function. It was also shown that Cd can cause lipid peroxidation in tissues, and that oxidative stress is probably one of the most important mechanisms of its toxicity including carcinogenicity<sup>21</sup>. On the other hand, there are still many doubts on oxidative stress induced by Cd as mechanism of toxicity, particularly since Cd does not produce reactive species directly<sup>22</sup>. Supportive evidences are: Cd in short time period start induces free radicals production, including superoxide radical, hydroxyl radical, hydrogen peroxide as well as free lipid radicals. Increase of lipid peroxidation is proven in lungs, brain and liver<sup>23</sup>, as well as blood<sup>11</sup> only few hours after exposure. Probably explanation for Cd induced oxidative damage could be inhibition of antioxidative defence system, mostly by decreasing glutathion concentration, or by interactions with the Fe, resulting in Fenton reaction and free radicals production<sup>11,22</sup>. Results of this study on Cd are in accordance with the literature. Additionally, even in vitro proven as potent prooxidative agent<sup>24</sup>, BDE-209, did not influenced Cd induced MDA production (Figure 1, Table 1). Based on results from Table 1, after effects assessment and confirmation of dose dependent manner, type of interaction between Cd and BDE-209 could be considered as additivity.



**Figure 1.** MDA concentration in *Wistar* rats kidney homogenates perorally exposed to Cd and/or 1000 mg BDE-209/kg/day during 28 days

One way ANOVA and post-hoc LSD test,  $p < 0.05$ : a – vs. DMSO group; b – vs. group receiving BDE-209; c – vs. groups receiving same dose of Cd.

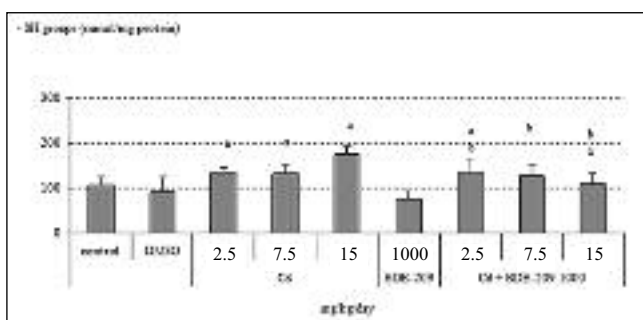
Observed decrease of SOD activity after increase of dose of Cd and/or BDE-209 in our study are contradictory with results from other studies where exposure was much longer<sup>2,12</sup>. It could be explained by application of different Cd doses, and different exposure duration. Decrease in SOD activity after Cd application observed in this experiment (Figure 2) could be explained by exhausting protective mechanisms or by up regulation of antioxidative enzymes activities. Dose-dependent manner was not conformed, therefore neither effect on SOD activity for the dose ranges (Table 1).



**Figure 2.** SOD activity in Wistar rats kidney homogenates perorally exposed to Cd and/or 1000 mg BDE-209/kg/day during 28 days

One way ANOVA and post-hoc LSD test,  $p < 0.05$ : a – vs. DMSO group; b – vs. group receiving BDE-209; c – vs. groups receiving same dose of Cd.

Important place in explanation of Cd toxicity mediated by oxidative stress take proteins and peptides reach in –SH groups, particularly methalotioneins (MT). Cadmium induces synthesis of MT, which play important role in transport, deposition and protection of Cd toxic effects<sup>20</sup>. In this experiment, increased concentrations of –SH groups were observed after exposure to Cd or to the combination of Cd and BDE-209. In our previous experiment we observed in vitro prooxidative effect of BDE-209, higher than one caused by Cd<sup>24,25</sup> which could be explanation for increase of –SH groups as adaptive response. For Cd is known that reacts with –SH groups, which could be explanation for decrease in this parameter, however it can damage protein structure, particularly by produced very toxic hydroxyl radical<sup>25</sup>. Literature data when rats were exposed to Cd by drinking water during 6, 12 or 24 weeks (3,17 - 4,28 mg/kg/day)<sup>26</sup> shown similar results like ones observed in our study.



**Figure 3.** –SH groups concentration in Wistar rats kidney homogenates perorally exposed to Cd and/or 1000 mg BDE-209/kg/day during 28 days

One way ANOVA and post-hoc LSD test,  $p < 0.05$ : a – vs. DMSO group; b – vs. group receiving BDE-209; c – vs. groups receiving same dose of Cd.

**Table 1.** Benchmark doses for Cd and Cd with BDE-209 as covariance for the effects on MDA concentration, SOD activity and –SH groups content in kidney homogenates

Effect on kidney		Cd (mg/kg/day)	Cd (mg/kg/day) - BDE209 covariance
		0	1000
MDA (nmol/mg protein)	CED	1.43	1.43
	CEDL5	1.008	1.008
	model	(+) E2	(+) E2
SOD activity (U/mg protein)	CED	/	/
	CEDL5	/	/
	model	(-) E2	(-) E2
-SH groups (nmol/mg protein)	CED	2.84	2.84
	CEDL5	1.839	1.839
	model	(+) E2	(+) E2

CED – Benchmark doza; CEDL5 – statistical lower confidence limit of Benchmark dose that cause change of 5% in effect; (-) no dose dependent effect; (+) dose dependent effect; En (n = 1, 2, 3, 4 ili 5) type of model dose – response, given in details in Slob (2002).

Observation from studies show that Cd after acute exposure inhibits enzymes of antioxidative protection i.e. SOD, and also decrease antioxidative capacity of tissue cells because of glutation depletion and –SH groups inactivation<sup>11,20,25</sup>. In study of Mladenovic et al. (2010)<sup>20</sup> dose of 2.5 mg Cd/kg caused after 24 hours significant increase of MDA, almost four folds higher SOD activity, since –SH groups content stayed at the control level. After prolonged exposure for 12 weeks Haouem and El Hani (2013)<sup>27</sup> showed that dose of Cd of 1.2 µg/kg/day and 132 µg/kg/day caused significant decrease in MDA while SOD and –SH groups were at the control levels.

**CONCLUSION**

Dynamic of oxidative process has influence on the parameters levels. Co-exposure with polybrominated organic pollutant did not influence Cd effects on oxidative stress parameters. Having in mind that this is pilot study, performed with only one dose of BDE-209, further investigations are necessary, including changes in dose ranges, duration of exposure etc. Moreover, results contributes to the issue of mixture toxicology even BDE-209 did not influenced oxidative stress as mechanism of Cd toxicity.

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## Sažetak

Poznato je da su bubrezi ciljni organ toksičnosti kadmijuma, ali je mnogo manje literaturnih podataka o toksičnim efektima BDE-209 na bubrege. Kadmijum, kao toksičan metal i BDE-209, kao organska halogenovana zagađujuća supstanca mogu imati sličan mehanizam dejstva posredovan oksidativnim stresom. Međutim, kako će Cd da deluje kao prooksidant u prisustvu BDE-209 još uvek nije proučavano, te je cilj ovog rada bio da se proceni uticaj BDE-209 na oksidativni stres posredovan kadmijumom. Rezultati ovog istraživanja ukazuju na blagi pad koncentracije MDA u homogenatima bubrega pacova nakon subakutne ekspozicije Cd i/ili BDE-209, dok se aktivnost SOD povećava i sadržaj -SH grupa smanjuje. Na osnovu procene odnosa doza-odgovor, BDE-209 nije imao uticaja na toksičnost Cd izazvanu posredstvom oksidativnog stresa (posmatrajući odabrane parametre). Potvrda je dobijena i iz izračunatih donjih granica pouzdanosti doze od 5% - CEDL<sup>5</sup> jer su vrednosti su bile iste za sam Cd i Cd primenjen u kombinaciji sa BDE-209. Može se zaključiti da dinamika oksidativnih procesa ima uticaj na vrednosti parametara oksidativnog stresa, da stoga istovremena ekspozicija Cd i polibromovanom organskom polutantu nije promenila uticaj samog Cd na parametre oksidativnog stresa. Imajući u vidu da je ovo pilot studija, izvršena sa samo jednom dozom BDE-209, potrebna su dalja istraživanja, uključujući i promene opsega doza, trajanje ekspozicije itd. Pored toga, rezultati ove studije doprinose tumačenju toksikologija smeša, iako BDE-209 nije imao uticaja na oksidativni stres kao mehanizam Cd toksičnosti za date uslove ekspozicije.

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