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RESEARCH ARTICLE

EFFECT OF CARBARYL ON THE BIOCHEMICAL RESPONSES OF THE INDIAN EARTHWORM *LAMPITO MAURITII*

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ABSTRACT

Earthworms are one of the major soil biota but the application of insecticides in the agricultural field has been threatened the soil beneficial organisms including earthworms. Hence, in the present study sublethal concentrations of carbaryl (T1-4.195ppm and T2-13.984ppm) on the biochemical response of the *L.mauritii* was investigated. The activities of following enzymes were measured: superoxide dismutase (SOD), catalase (CAT), glutathione-s-transferase (GST) and acetyl cholinesterase (AChE). The levels of lipid peroxides (LP) and glutathione level (GSH) were also determined. The results showed that the GSH, SOD, CAT, GST and AChE activities were decreased than the control up to 15 days thereafter slightly increased on the 30 days. The results suggested that carbaryl was affected the earthworm populations in the soil.

INTRODUCTION

In developing countries like India where the economy depends largely on agricultural products, 15-20% of harvest is destroyed due to pests and uncontrolled use of pesticides by the Indian farmers. This results in rapid increase of soil contamination by pesticide residues. Carbaryl is an organophosphate pesticide. It is commonly used in agricultural field against insect pests because it is beneficial for soil organisms. The eco-toxicity studies concerning the effects of pesticides on soil organisms are needed in order to evaluate their toxicity and to prevent the potential risk of their environmental pollutants on the terrestrial ecosystems. Earthworms are important organisms in the soil and they appear to be the best organisms for use in soil toxicity evaluation (Bouche, 1992). Earthworms represent the greater fraction of biomass of invertebrates in ground (>80%). They are playing a variety of important roles in agro ecosystems like feeding and burrowing activities, incorporate organic residues and amendments into the soil, enhancing decomposition, humus formation, nutrient cycling, and soil structural development. For these reasons, earthworms have gained acceptance for use in tests to assess the effect of chemicals on soil organisms. Several earthworms protocols have been developed to assess the effects of chemicals on earthworms among which the most well known is the OECD guideline 207 (OECD, 1984). Enzyme assay in earthworms being developed to give a measure of pesticides exposure and act as a suitable biomarker (Booth *et al*., 1998). The activities of enzymes such as oxidoreductases (eg. Superoxide dismutase and catalase), transferases (glutathione-s-transferase), hydrolases (eg. acetylcholinesterase) in earthworms are regarded as fast and prognostic indices of the environmental stress (Scaps *et al*., 1997; Saint Denis *et al*., 1998 and 1999; Booth *et al.,* 2000).

The formation of highly reactive oxygen species (ROSs) is a normal consequence of a variety of essential biochemical reactions including mitochondrial and microsomal electron transport systems, phagocytosis, xenobiotic-enhanced redox cycling and transition metal chemistry (Halliwell and Gutteridge, 1990). As a consequence of the instability of these ROSs and their potential to damage cells and tissues, there are both enzymes (SOD, CAT and GST) and small molecular weight molecules (GSH) with antioxidant capabilities that can protect against the adverse effects of ROS reactions (Saint-Denis *et al*., 1998). Pesticides including carbamates, organochlorides and organophosphorus compounds have been shown to alter lipid metabolism in fish or invertebrates (Rao and Rao, 1979; Coglianese and Neff, 1982; Rajyalakshmi and Reddy, 1988). Ribera *et al.* (2001) had studied the effect of carbaryl on CAT, AChE, GST and GSH of *Eisenia fetida*. Rannug and Rannug (1984) found that dithiocarbamates inhibited enzymes involved in the defence against free radicals such as SOD, CAT and GST of *Salmonella typhimurium.* The effect of carbaryl on AChE activity of *E. andrei* have been studied by Caselli *et al.* (2006).

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The present paper reports the effect of organocarbamate insecticide, carbaryl on the biochemical analysis (LPX, GSH, SOD, CAT, GST and AChE) of agriculturally important Indian earthworm *L. mauritii*.

MATERIALS AND METHODS

Chemicals

The following chemicals were purchased from Sigma-Aldrich chemicals Co. (USA): reduced glutathione (GSH), hydrogen peroxide (H_2O_2), 1-chloro-2,4-dinitrobenzene (CDNB), phenazinemethosulphate (PMS), nitrobluetetrasolium (NBT), nicotinamide adenine dinucleotide (NADH), acetylthiocholine iodide (AThch), 5,5' ethylene diamine tetra acetic acid (EDTA). All other chemicals used were of analytical grade.

Earthworms

The earthworm *L.mauritii,* clay loam soil and fresh cow dung were obtained from Annamalai University, agricultural farm and dairy-yard. Matured and healthy earthworms were acclimatized under laboratory conditions in cow dung substrate for one week. They were kept at room temperature 28±2°C with 50-60% moisture. The earthworms used in this assay were adults with well developed clitellae. The earthworms were removed from the culture medium 24h before use and stored in petri dishes on damp filter paper to void gut contents.

Toxicity test

Based on the acute toxicity evaluation, lower and higher sublethal concentrations for carbaryl (T1-4.195ppm and T2-13.984ppm) have been selected from 96h LC50 values. Five earthworms were introduced into each sublethal concentration of carbaryl mixed soil substrate and control. The control was treated with only water. For the enzyme assay, *L.mauritii* were collected from the treatments and control (five replicates) at the end of 1,5,15 and 30 day of experiment as followed by many earlier researchers (Booth *et al*., 1998 and Ribera *et al.,* 2001). The earthworms were placed in Petridis on filter paper and kept in the dark for 24h to void gut contents. After 24h, earthworms were homogenized for 1 min in Tris buffer for protein and enzyme analysis (Ribera *et al.,* 2001).

Biochemical assays

Lowery *et al*. (1951) method was followed to estimate the protein content in the earthworm's tissue homogenate. Lipid peroxidation was estimated by the formation of thiobarbituric acid reactive substances (TBARS) and quantified in terms of malondialdihyde (MDA) equalents according to the method described by Nichens and Samuelson (1968). Ellman (1959) method was used to estimate reduced glutathione (GSH). Superoxide dismutase (SOD) was assayed according to the method of Kakkar *et al*. (1984). Sinha (1972) method was used to determine the activity of catalase. The glutathione-S-transferase activity was measured in tissue homogenate by following the increase in absorbance at 340 nm in spectromic-20 (Bausch & lamb) using 1-chloro-2,4-dinitrobenzene as a substrate (Habig *et al.,*1974). Acetylcholinesterase (AChE) activity was determined according to methods adapted from Ellman *et al.* (1961).

Statistical analysis

The statistical significance of the data was tested by using Dunnett's multiple range test and two way analysis of variance.

RESULTS

In control, the level of LPX, GSH, SOD, CAT, GST and AChE activity was almost similar in all the periods of experiment. The LPX level was significantly increased over control in both T1 and T2 respectively on 1,5 and 15 days (T1-11%, 26% & 14% ; T2-28%, 41% & 25%). On the day 30, the LP level was insignificantly decreased (3%&9%). GSH level in T1 and T2 was significantly decreased on 1st, 5th and 15th day over control (T1-7%, -11% & -10%; T2-10%, 10% & -11%). On 30th day, the GSH level was slowly increased (-3%&-9%). SOD activity in T1 and T2 was significantly decreased over control on 1st, 5th and 15th days (T1-18%, -27% & -47%; T2-26%, -35% & -50%) thereafter slowly increased on 30th day(-14%&-24%). In T1 and T2, CAT activity was significantly decreased about on 1st, 5th and 15th d over control (T1-31%, -42% & -51%; T2-42%, -42% & -60%). On 30th day, CAT activity was slowly increased (-10%&-18%). GST activity was significantly decreased over control in T1 and T2

Table 1. Biochemical responses of *L.mauritii* exposed to Carbaryl mixed soil substrate

Biochemical Measurements	Treat Ments	Duration of exposure (days)			
		1	5	15	30
Lipid peroxidation(LP) (nmol MDA mg-1 protein)	C	0.36±0.01	0.34±0.02	0.36±0.02	0.34±0.03
	T1	0.40±0.03* (+11.11)	0.43±0.01* (+26.47)	0.41±0.03* (+13.89)	0.35±0.02^{NS} (+2.94)
	T2	0.46±0.03* (+27.78)	0.48±0.02* (+41.18)	0.45±0.04* (+25.00)	0.37±0.01^{NS} (+8.82)
Reduced glutathione(GSH) (µg g^{-1} Fw)	C	1.35±0.09	1.34±0.08	1.31±0.07	1.34±0.06
	T1	1.25±0.06* (-7.41)	1.19±0.04* (-11.19)	1.18±0.05* (-9.92)	1.21±0.07* (-9.70)
	T2	1.21±0.04* (-10.37)	1.21±0.05* (-9.70)	1.16±0.07* (-11.45)	1.19±0.05* (-11.19)
Superoxide dismutase(SOD) (µmol mg^{-1} protein min^{-1})	C	28.28±0.88	25.21±0.69	27.28±1.15	29.29±0.95
	T1	23.13±0.78* (-18.21)	18.51±0.37* (-26.58)	14.50±0.65 (-46.85)	25.08±0.84* (-14.37)
	T2	20.83±0.81* (-26.34)	16.47±0.36* (-34.67)	13.55±0.70* (-50.33)	22.25±0.38* (-24.04)
Catalase(CAT) (µmol mg^{-1} protein min^{-1})	C	47.32±2.91	49.64±2.11	50.87±1.74	46.11±2.13
	T1	39.54±1.69* (-16.44)	34.67±1.38* (-30.16)	30.34±0.97* (-40.36)	41.47±1.26* (-10.06)
	T2	35.53±1.25* (-24.92)	31.45±0.71* (-36.64)	27.43±0.76 (-46.08)	37.86±0.48* (-17.89)
Glutathione-S-transferase(GST) (µmol mg^{-1} protein min^{-1})	C	237.37±5.25	244.36±4.34	243.74±6.08	236.34±4.87
	T1	178.50±5.05* (-24.80)	145.36±2.91* (-40.57)	108.91±3.27* (-55.32)	155.89±3.16* (-34.04)
	T2	156.26±3.14* (-34.17)	130.84±3.42* (-46.46)	93.79±2.55* (-61.52)	147.01±3.08* (-37.80)
Acetylcholinesterase (AChE) (nmol mg^{-1} protein min^{-1})	C	65.58±2.02	60.28±1.31	62.65±2.06	64.37±2.63
	T1	15.62±0.53* (-76.18)	12.56±1.54* (-79.16)	10.40±0.77* (-83.40)	11.59±1.32* (-81.99)
	T2	13.59±0.69* (-79.28)	11.59±0.72* (-80.77)	9.26±0.58* (-85.22)	9.69±0.58* (-84.95)

Result are expressed as mean±standard error of five observations % change over control values are given in parenthesis *- Significant at 5% level (P< 0.05 for comparisons with control) NS – Not Significant (P>0.05) (based on Dunnett's test)

on 1st, 5th and 15th d (T1-25%, -41% & -55% ; T2-34%, -46% & 62%). On 30th day, the GST activity was slowly increased (-34%&-38%). AChE activity in T1 and T2 was significantly decreased on 1st, 5th and 15th day over control (T1-76%, -79% & -83%; T2-79%, -81% & -85%). On 30th day, AChE activity was almost equal to 15th d activity increased. The inhibitory effect of carbaryl on the biochemical parameters were more in higher concentration than lower concentration. The results indicated that compared with control, carbaryl exert inhibitory effect on *L.mauritii.*

DISCUSSION

In earthworms, pesticides toxicity leads to oxidative stress due to production of ROSs which in turn increased lipid peroxidation (LPX) brought out changes in GSH, antioxidant enzymes (SOD, CAT, GST) and inhibit AChE activity. Productions of free radicals and oxidative stress have been commonly known to occur during exposure of animals to many factors including pesticides (Bagchi *et al*., 1995). Oxidative stress can damage many biological molecules like proteins, DNA and lipids. Hence, biological membranes are also among the main targets for free radicals. Superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione-S-transferase (GST) constitute a mutually supportive team of defense against reactive oxygen species. High LPX level and low level of GSH, antioxidant enzymes (SOD, CAT, GST) and AChE activities were observed in T1 than T2.

This observation indicated that a dose dependant impact on earthworms. Similar dose dependent impact of pesticides on earthworms was reported by many researchers (Davey, 1963; Martin, 1976; Yajuan Shi *et al*., 2007). In experiment, LPX level was increased than the control up to 15 days afterwards declined due to reduction of LPX level. The GSH level, SOD, CAT, GST and AChE activities were progressively decreased up to 15 days of carbaryl exposure thereafter slightly increased at 30th day. The results suggested that the toxic effect of carbaryl was more pronounced up to 15 days due to presence of pesticide residues and recovery from the impact after 15 days due to biodegradation or detoxification of carbaryl. Mosleh *et al*. (2005) exposed an aquatic worm *Tubifex* to isoproturon herbicide and observed disappearance of herbicide due to degradation as indicated by the appearance of metabolite in water.

The exposure of earthworms to sublethal concentrations of endosulfan, monocrotophos and carbaryl caused oxidative stress through chemical reactions producing hydroxyl radical species which are believed to initiate lipid peroxidation (Yiin *et al*., 1999). Bagchi *et al*. (1995) reported that cadmium and pesticides caused GSH depletion by stimulating the free radical production. The depletion of glutathione level in the present experiment is thus one of the factors responsible for enhanced lipid peroxidation. The present results are correlated well with other reports, where pesticides have been shown to reduce GSH level in earthworms. Saint-Denis *et al*. (1999) observed that LPX was increased in *E. fetida* after 1 and 7 d of benzopyrene exposure. They were also observed that declined total glutathione studied the effect of benzo (a) pyrene on the biochemical responses of the earthworm, *Eisenia fetida* and reported that the total glutathione concentration declined after 1 and 2 d exposure. Rannug and Rannug (1984) found that dithiocarbamates inhibited enzymes involved in the defense against free radicals such as SOD and CAT. Imidacloprid inhibited the SOD activity of *E. fetida* (Luo *et al*., 1999). GST also known as detoxifying enzyme, which promote the conjugation of reduced glutathione with a variety of reactive electrophilic compounds resulting to the formation of less toxic substances which are easily excreted from the body (Chaseaud, 1979). Ribera *et al*. (2001) reported the activity of glutathione-S-transferase (GST) in *E. fetida* was decreased after 2 and 14 days exposure of carbaryl. Hans *et al*. (1993) showed that total GST activity in the earthworm *Pheretima posthuma* declined considerably in the early stages of exposure to aldrin and endosulfan thereafter GST activity subsequently increased to near control levels after 4 weeks.

The organophosphorus and carbamate insecticides are known to inhibit acetylcholinesterase which plays an important role in neuro transmission at cholinergic synapses by rapid hydrolysis of neurotransmitter acetylcholine to choline and acetate (Kwong, 2002; Kavitha and Venkateswara Rao, 2007). The maximum AChE inhibition was observed in *Drawida willsi* after 12 days carbofuran exposure (Panda and Sahu, 2004). AChE activity has been shown to be strongly inhibited by organophosphates and carbamates in a number of invertebrate species (Day and Scott, 1990). Caselli *et al.* (2006) reported that carbaryl inhibited the AChE activity of *Eisenia andrei.* Carbofuran treated worms exhibited maximum inhibition in the AChE activity, as compared to malathion and butachlor pesticides. The inhibition was maximum after 12 days, being 55% and 63% in the worms treated with single and double doses of carbofuran over control respectively (Panda and Sahu, 2004). Ribera *et al*. (2001) reported that in *Eisenia fetida*, acetylcholinesterase activity was strongly inhibited by carbaryl, continuously decreased to 87% of the control value at all doses (12, 25 and 50 mg kg^{-1} soil) for the period of 2, 7 and 14 days. They concluded that AChE inhibition was observed even at the lowest dose of carbaryl and the shortest duration of exposure and the residual activity was stable whatever the dose or the exposure duration. Pradhan and Mishra (1998) studied the inhibition and recovery kinetics of acetylcholinesterase activity in *Drawida calebi* and *Octochaetona surensis* exposed to carbaryl insecticide and observed inhibition of the enzyme activity in both species within 24 hrs of exposure with maximum inhibition at 9th day.

Conclusion

The enzyme assay clearly proved that carbaryl intoxicated *L.mauritii* showed elevated level of LP and lesser activity of antioxidants up to 15 days. After 15 days the reverse trend occurred due to the pesticide biodegradation but not to reach the control level. AChE activity is highly inhibited by carbaryl than other pesticides. The expriment proved that carbaryl inhibited the enzyme activity of earthworms.

REFERENCES

Bagchi, D., Bagchi, M., Hassoun, E.A., and Stohs, S.J. 1995. In vitro and in vivo generation of reactive species, DNA

damage and lactate dehydrogenase leakage by selected pesticides. *Toxicology*, 104:129-140.

Booth, L.H., Heppelthwaite, V.J. and O' Halloran, K. 2000. Growth, development and fecundity of the earthworm *Aporrectodea Caliginosa* after exposure to two organophosphates. *New Zealand plant production*, 53: 221-225.

Bouche, M.B. 1992. Earthworm species and ecotoxicological studies. In Greig-Smith PW, Becker H, Edwards PJ and Heimbach eds., Ecotoxicology of earthworms. Intercept, Andover, UK pp-20

Caselli, F., Gastaldib, L., Gambia, N., and Fabbria, E. 2006. In vitro characterization of cholinesterases in the earthworm Eisenia andrei. Comp. Biochem. *Physiol. C Toxicol. Pharmacol*, 143: 416–421.

Chaseaud, L.F. 1979. The role of glutathione and glutathione-S-transferase in the chemical carcinogens and other electriphillic agens. *Adv. Cancer Res*., 29: 175-274.

Coglianese, M.P. and Neff, J.M. 1982. Biochemical response of blue crab, callinectes sapidus, to pentachlorophenol. In: vernberg, W.B., Calabrese, A., Thurberg, F.P., Thurberg, F.J., (Eds.). Physiological mechanisms of marine pollutant toxicity. Academic press, New York, pp. 127-143.

Davey, S.P. 1963. Effects of chemicals on earthworms: a review of the literature. Special scientific Report-Wildlife, No 74. U.S. Department of the Interior Washington.

Day, K.E. and Scott, I. M. 1990. Use of acetylcholinesterase activity to detect sublethal toxicity in stream invertebrates exposed to low concentrations of organophosphate insecticides. *Aquatic toxicol*, 18: 101-114.

Ellman, G.C. 1959. Tissue sulfhydys groups. *Arch Biochem Biophys*, 82: 70-77.

Ellman, G.L., Courtney, K.D., Andres, V., and Feather S.R.M.. 1961. A new and rapid colorimetric dtermination of acetyl cholinesterase activities. *Biochem pharmacol*, 7: 88-95.

Habig, W.H., Pabst, M., and Jackoby, W.B.C. 1974. Glutathione S-transfers. First enzymatic step in mercapturic acid formation. *J. Biol. Chem*., 249: 7130-7139.

Halliwell, B., and Gutteridge, J.M.C. 1990. Role of free radicals and catalytic metals ions in human disease: an overview. *Method Enzymol*, 186B: 1-85.

Hans, R.K., Khan, M.A., Farooq, M. and Beg, M.U. 1993. Glutathione-s-transferase activity in an earthworm *pheretima posthuma)* exposed to three insecticides. *Soil Biol. Biochem*, 25: 509-511.

Kakkar, P., Das, B., and Viswanathan, P.N. 1984. A modified spectro photometric assay of SOD. *Indian J. Biochem. Biophys*, 21:130-132.

Kavitha, P., and Venkateswara R.J. 2007. Oxidative stress and locomotor behaviour response as biomarkers for assessing recovery status of mosquito fish (Gambused affinis) after lethal effect of an argonophosphate pesticide, monocrotophos, *Pestic. Biochem. Phisiol*, 87(2): 182-188.

Kwong, T.C. 2002. Organophosphate pesticides: biochemtry and clinical toxicology. *Ther. Drug. Monit*, 24: 144-149.

Booth, L.H., Heppelthwaite, V., and Eason, C.T. 1998. Cholinesterase and glutathione S-Transfrase in the earthworm apporectodea caliginosa as Biomarkers of organophophate exposure. Proc. 51st N.Z. plant protection Conf, pp-138-142.

Lowry, O.H., Rosebrough, N.J., Farand, A.L., and Randall, R.J. 1951. Protein measurement with a folin phenol reagent. *Journal biological chemistry*, 193: 265-275.

Luo, Y., Zang, Y., Yaun, Z. and Zhiming, K. 1999. Toxicological study of two novel pesticides on earthworm Eisenia foetida. *Chemosphere*, 39(13): 2347-2356.

Martin N.A. 1976. Effect of four insecticides on the pasture ecosystem. V. Earthworms (Oligochaeta: Lumbricidae) and arhtropoda extracted by wet sieving and salf flotation. *New Zealand Journal of Agricultural Research*, 19: 111-115.

Mosleh, Y.Y. Paris-Palacios, S., Couderchet, M., Biagianti-Risbourg, S., and Vernet, G. 2005. Effects of the herbicide isoproturon on metallothioneins, growth, and antioxidative defenses in the aquatic wormTubifex tubifex (Oligochaeta, Tubificidae). *Ecotoxicology*, 14(5): 559–571.

Nichens, W.C.J., and Samuelson, B. 1968. Formulation of malondialdehyde from phospholipid arachidonate during microsomal lipid peroxidation. *Eur. J. Biochem*, 6:126-130.

Organization for Economic Cooperation and Development. 1984. Earthworm acute toxicity tests. Chemicals testing guideline 207. OECD publications, Paris, France.

Panda, S. and Sahu, S.K. 2004. Recovery of acetylehdine esterase activity of *Drawida Willsi* (oligochacta) following application of three pesticides to soil. Chemosphere, 55F: 283-290.

Rajyalakshmi, A., and Reddy, T.G. 1988. Effect of carbaryl and lindane on the lipid content of maternal tissues and embryos of the viviparous scorpin, Heterometrus fulvips (koch). *Journal of Environmental Biology*, 9: 97-105.

Rannug, A., and Rannug, U. 1984. Enzyme inhibition as possible mechanism of the mutagenicity of dithiocarbamic acid derivatives in salmonella typhimurium. *Chemico-Biological Interactions,* 49: 329-340.

Rao, K.S.P., and Rao, K.V.R. 1979. Effects of sublethal concentration of methylparathion on selected oxidative enzymes and organic constituents in the tissue of the freshwater fish; Tilapia massambica (petry). *Current Science*, 48: 526-528.

Ribera, D., Narbonne, J.F. Arnaud, C, and Saubt-Denis, M. 2001. Biochemical response of earthworms *Eisenia fetida* exposed to contaminated artificial soil, effects of carbaryl. *Soil Biol. Biochem*, 33: 1123-30.

Pradhan, S and Mishra, P.C. 1998. Inhibition and Recovery kinetics o fAcetylcholinesterase activity in *Drawida Calebi* and insecticide. *Ball. Environ. Contam. Toxicol*, 60: 904-908.

Saint – Denis, M., Narbonne, J.F., Arnaud, C., and Ribera,D. 1999. Biochemical responses of the earthworm Eisenia fetida anderi exposed to contaminated artificial soil, effects of benzo(a)pyrene. *Soil Biology & Biochemistry*, 31: 1837-1846.

Saint-Denis, M., Labrot, F., Narbonne, J.F., and Ribera, D. 1998. Glutathione and glutathione related enzymes, and catalase activities in the earthworm *Eisenia fetida Andrei. Arch. Environ. Contam. Toxicol*, 35: 602-614.

Scaps, P., Demuynck, S., Descamps, M., and Dhainaut, A. 1997. Effects of organophosphate and carbamate pesticides on acetylcholinesterase and choline acetyltransferase activities of the polychaete *Nereis diversicolor. Arch. Environ. Contam. Toxicol*, 33: 203-208.

Sinha KA. 1972. colorimetric assay of catalase. Ann Biochem, 47: 389-394.

Yajuan, S., Yajing, S., Xin W. and Yonglong Lu, S.Y. 2007. Comparative effects of Lindane and deltamethrin on mortality, growth, and cellulose activity in earthworms (Eisenia fetida) pesticide Biochemistry and physiology, 89: 31-38.

Yiin, S.J., Chan, C.L., Sheu, J.Y., Tseng., W.C., and Lin, T.FH. 1999. Cadmium induced Lipid peroxidation in rat testes and protection by selenium. *Biometals*, 12: 353-359

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