Supplementary information

Occurrence, fate, effects, and risks of dexamethasone: ecological implications post-COVID-19

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Table S1. The ecotoxicological effects of DEXA on organisms at different levels of organization

DEXA Properties	**Organism type**	**Organism**	**Exposure concentration**	**Exposure conditions**	**Duration**	**Effects studied (Endpoints)**	**Effects observed**	**NOEC, LC_{50}/EC_{50}**	**Refs**
DEXA	Microbial community	Bacteria	500 ng/L	Aquifer media, Beijing Chaobai River (BJ), Hebei Hutuo River (HB), and Tianjin Duliujian River water samples	50 d	Microbial community changes	DEXA completely degraded, with removal rates varying due to microbial diversity and composition	NR	[1]
Irradiated and non-irradiated DEXA	*Photobacterium phosphorium*	Bacteria	NR	2% NaCl solution	15 min	Bioluminescence test	The solution containing DEXA was more toxic than DEXA following irradiation for 24 h	N.D before irradiation, EC_{50} =133.80 mg/L following irradiation	[2]

DEXA and TPs	*Pseudokircheneriella subcapitata*	Algae	NR	ISO procedure 8692, under continuous illumination of 8000 lux at 25 ± 1 °C	72 h	Growth inhibition	DEXA had no effect on algal growth, while TPs inhibited algal growth	EC_{50} = 12.15, 40.75, > 100 mg/L, for TP11, TP12 and DEXA, respectively	[3]
Non-irradiated DEXA 4 000 gamma-irradiated DEXA	*Microcystis flos-aquae* *Scenedesmus obliquus*	Algae	25, 50 and 100 mg/L	BG 11 medium, light/dark cycle of 12 h/12 h, at 25 ± 1 °C	14 d	-Algal growth -Chlorophyll-a content	-Non-irradiated DEXA induced concentration-dependent algal growth promotion, and increase chlorophyll-a content for both algal species - For radiated DEXA, chlorophyll-a content of *M. flos-aquae* increased by 4.27%, but decrease by 25.65% for *S. obliquus*	NR	[4]

DEXA and TPs	*Brachionus calyciflorus*	Rotifer	NR	Moderately hard medium EPA, Hardness was 80–100 mg/l $CaCO_3$, dissolved oxygen content was at least 90%, 25 ± 1 °C in the dark	24 h	Mortality	TPs were more toxic than parent DEXA	LC_{50}= 13.20, 44.66 and 48.22 mg/L, respectively for TP 11, TP 12 and DEXA	[3]
DEXA and TPs	*Thamnocephalus platyurus*	Crustacean	NR	Moderately hard EPA medium, 25 ± 1 °C in the dark	24 h	Mortality	TPs were more toxic than parent DEXA	LC_{50}= 20.9, 30.52 and 60.11 mg/L, respectively for TP 11, TP 12 and DEXA	[3]
DEXA and TPs	*Daphnia magna*	Crustacean	NR	Aerated synthetic reconstituted, hardness 250 mg/l expressed as	24 h	Immobilization	TPs were more toxic than parent DEXA	EC_{50}= 10.88, 17.82, and 48.30,mg/L, respectively,	[3]

				$CaCO_3$, 20 ± 1 °C in the dark freshwater				for TP 11,TP 12 and DEXA	
DEXA and TPs	*Ceriodaphnia dubia*	Crustacean	NR	Synthetic reconstituted aerated hard ISO medium, hardness 250 mg/l as $CaCO_3$, at 25 ± 1 °C with a 16:8-h light: dark cycle (500 lux).	7 d	Population growth inhibition	TPs were more toxic than parent DEXA	EC_{50} = 0.05, 0.13, and 0.06 mg/L for DEXA, TP 11 and TP 12, respectively	[3]
DEXA	*Ceriodaphnia dubia*	Crustacean	0.05 to 3.2 mg/L	Moderately hard reconstituted water	48 h	Immobilization	Immobility was observed	EC_{50} = 0.75 mg/L	[5]
DEXA	*Ceriodaphnia dubia*	Crustacean	1.95-125 µg/L	Moderately hard reconstituted water	NR	Multigenerational chronic bioassays	Significant reduction in reproduction was observed at the	NR	[5]

							lowest concentration of 3.9 µg/L		
DEXA	Rainbow trout *(Oncorhynchus mykiss)*, (hepatic microsomes)	Fish	0.39, 19.62 mg/L	Freshwater 14±1°C and DEXA prepared in DMSO and METOH and exposure at 21°C	10 min	Fish CYP1A-enzyme activity (EROD)	No effect on EROD activity	NR	[6]
DEXA with indole-3-carbinol	Rainbow trout (hepatic microsones)	Fish	0.39, 19.62 mg/L	Freshwater 14±1°C and DEXA prepared in DMSO and METOH and exposure at 21°C	10 min	Fish CYP1A-enzyme activity (EROD)	EROD activity declined	NR	[6]
Betamethasone	Japanese medaka	Fish	0.01, 0.1, 1.0 µg/L used in two-generation	Continuous flow through diluter system/water	0-133 d	-Secondary sexual characterisation, -vitellogenin expression	-Wet weight was low -Endocrine related disruptions developed	NOEC = 0.1 µg/L	[7]

			fish full life cycle				-Lifetime exposures resulted in low reproductive success, -3-fold increase in vitellogenin expression in males at 1.0 µg/L		
DEXA	Adult *Hoplias malabaricus*	Fish	0, 0.03, 0.3, or 3 µg/kg	Filtered and dechlorinated tap water. Trophic exposure light/dark cycle of 12h/12h at 24±1 ºC	30 d	- Oxidative stress - Biotransformation in liver and gonads - Endocrine disruption	No effect of EROD activity	NR	[8]
DEXA	*Sparus aurata L.*	Fish	800 mol/m^3	DEXA administered through food pellets	35 d	- Intermediary metabolism - Growth	- Lower growth rates - Lower body mass		[9]

- High hepatosomatic index (HIS)
- Reduced plasma cortisol levels
- Reduced TAG
- High acid levels
- Increased oxidation in both liver and muscles
- High GPDH (glucose metabolism)
- Enhanced hepatic storage
- Higher GLDH and LDH activities in muscles
- Plasma osmolality higher

DEXA	*Damio rerio* larvae and adult male fish	Fish	50/500 mg/L 5/50 mg/L	E3 medium for 120 h at 28 ± 0.5 ºC 12 h at 29 ± 1.0 ºC	4 d	Glucocorticoid responsive genes	- Unregulated expression of *pepck, biap2* in zebrafish larvae - *Baiap2, prx*, and *mmp2* unregulated in adult zebrafish	EC_{50} =1.8nM	[10]
DEXA	Male and female juvenile zebrafish	Fish	-0.1, 0.2, 4 and 15.8 mg/L	Egg water at 28°C 14 h light/10 h darkness cycle, water and air at 4 °C and 23 °C, respectively Eggs:13 h light/11 h dark at 28 °C	All exposed from 1 h-48 h and 48 h – 96 h post infections	- dynamics of glucocorticoid uptake by zebrafish chlorinated embryos	-uptake dependent on developmental stages of the embryo	NR	[11]

DEXA	-Rainbow trout -Mummichog killifish (*Fundulus heteroclitus*)	Fish	50, 100 mg/kg (trout) 100 mg/kg (killifish)	Killfish at 19-20°C on recirculating sea water Trout at 12-15°C in flow-through water	48 h	Metabolic rates	· Killifish exhibited higher metabolic rates · DEXA induced 3-cyano-7-ethoxycoumarin metabolism	NR	[12]
DEXA	Fathead Minnow *(Pimephales promelas)*	Fish	62.5, 125, 250, 500, 1000 µg/L	Dimethylformamide or METOH 16 h light/8 h dark at 28 °C ± 1 °C	28 d	Survival and growth	-decreased survival -growth not impacted	LC$_{50}$=254 µg/L NOEC= 254 µg/L, and LOEC=577 µg/L	[13]
DEXA	Fathead Minnow	Fish	0.1, 50, 500 µg/L	Continuous flow-thru exposures conducted by pumping diluted DEXA at 25±1°C with a photoperiod of 16	-21 d reproduction study -29 d embryo-larvae	-reproductive toxicity and early life effects	-500 µg/L exposure at 21 d caused: -reduction in fecundity, female plasma estradiol, increase in plasma	NR	[14]

				h light and 8 h dark.			vitellogenin protein levels -500 µg/L exposure at 29 d resulted in deformed gill opercula, reduction in weight and length		
DEXA	Crucian Carp (*Cyprinus carpio*)	Fish	20 mg/L	Dechlorinated water at 25°C	1, 2, 4, 6, 8, 10 d	-effects on regeneration process in caudal fin	-smaller fins -regeneration delayed but no apparent inhibition	NR	[15]
DEXA	Rainbow trout	Fish	0.39, 3.93, 19.62 and 39.25 mg/L	Freshwater	5, 10, 30 min	-Inhibitory effects of Dexa on 3 CYP450 mediated activities; (EROD, CYP1A) (BFC-OD, CYP3A) (PNPH, CYP2E1)	-no inhibitory potency on any enzyme	NR	[16]

DEXA	Rainbow trout (juvenile)	Fish	3, 30 300 and 3000 ng/L	Freshwater	21 and 42 d	-effects on CYP450 system on fish	-no change in CYP450 activities -induction of the CYP3A protein observed at the lowest concentration	NR	[16]
DEXA	Crucian carp (*Carassius auratus*)	Fish	200 µg/kg	NR	0, 3, 6 and 9 d	- effects on the immune response of DEXA treated fish infected with *Aeromonas hydrophila*	- fish exposed to DEXA and then infected with a pathogen, had higher mortality -Immune gene expression *il-1β, cxcl-8*, *tnfα*, and *crp* down-regulated	NR	[17]
DEXA	Sterlet (*Acipenser ruthensus L.*)		0.8 mg/ml	Aerated tanks at 16-18°C, fish exposed to DEXA in Ringers solution	0, 1, 3, 7, 14 and 21 d	- effects of dexa on the oxidative processes in the immunocompete	- activation of lipid peroxidation	NR	[18]

						nt organs (liver, kidney and spleen)	-decrease in antioxidant activity		
DEXA	Adult Zebrafish	Fish	2 and 20 mg/l	Freshwater. 14 h light and 10 h dark.	24 h	- effects of stress on behaviour, melatonin and stress-related gene expression (*nfkb)*	-anxious behaviour, lower level of melatonin, higher mRNA expression of *nfkb*	NR	[19]
DEXA and *Piper betle L.* (PB) leaf extract and Melatonin	Adult Zebrafish	Fish	-Melatonin (10mg/kg) - *Piper betle L.*(30mg/kg) exposed to fish after DEXA treatment	Freshwater. 14 h light and 10 h dark.	24 h	- effects on behaviour and expression of - levels of melatonin related genes (*MTI, MT2, aanat1, aanat2*) and stress-related (*nfkb*) gene	-PB enhanced sleep behaviour in fish -*aanat2* suppression on PB, melatonin and DEXA treated fish -*MT2* increased expression on PB, melatonin and DEXA	NR	[19]

							-no change in the *MT1* and *aanat1* expression. -suppressed *nfkb* mRNA expression		
DEXA	Crucian Carp	Fish	1mg/L	Dechlorinated tap water at 25-26°C. 14h light: 10h dark;	35 d	- assessing immunomodulatory effects of chemical pollutants on fish using DEXA followed by exposure to *Aeromonas salmonicida*	-100% mortality on bacteria-infected fish exposed to DEXA - lower leukocytes and haemolytic complement activity	NR	[20]

NR-not reported

Table S2. Removal of DEXA during wastewater treatment in WWTPs with techniques utilized across geographical regions globally in selected studies.

Location	**Technology**	**Water Type (Environmental matrix)**	**Influent conc.**	**Effluent conc.**	**%Removal**	**Sludge**	**Reference**
Italy	Adsorption Modified clay / GAC	Dexamethasone sodium phosphate (DSP) from Wastewater	100 mg/L		GAC (35.7%), Clay (< LQL) (Outlet)		[21]
Brazil	Electrocoagulation	Hospital wastewater	0.1 mg/L		38%		[22] [23]
Croatia	Adsorption Waste Sludge	Industrial Wastewater	80 mg/L		80%		[24]
India	Electro coagulation	Synthetic wastewater	1 400 mg/L		86%		[25] [4]

Iran	Photocatalysis ZrO_2 and WO_3	Synthetic wastewater	5 - 50 mg/L		20 – 100%	[4]
Iran	Photocatalysis Ag/TiO_2 visible light	Synthetic wastewater	15 mg/L		30 – 70%	[26]
Iran	Sono-nanocatalysis	Synthetic wastewater	15 – 45 mg/L		60 – 90 %	[27]
Croatia	Membranes NF and RO	Synthetic wastewater	10 mg/L		> 90%	[28] [29]
Switzerland	Membrane bioreactor	Hospital Wastewater	147 ng/L	ND	100%	[30]
USA	Ozonation	Synthetic wastewater	2 mg/L		60%	[31]
Italy	Photocatalysis TiO_2–UV light	Synthetic wastewater	10 mg/L		100%	[32]

	Adsorption Modified clinoptilolite zeolite		5 – 40 mg/L		35 – 80%		[33], [119]
Spain	Adsorption Montmorillonite	Synthetic wastewater	50 mg/L		90.6%		[21]
China	Gamma irradiation	Synthetic wastewater	2 – 50 mg/L		95 – 100%		[34]
	Lagoons	Swine wastewater	260 ng/L	37.8 ng/L	85%	35.0 ng/g	[35]
	Biological WWT	WW influent (Huiyang WWTP)	22.6 ng/L	ND*	100%	ND	[36]
		WW influent (Meihu WWTP)	3.8 ng/L	ND*	100%	ND	
USA	AOP and Membranes Ozone, UV, RO, MF	WW effluent	0.11–0.16 ng/L	< 0.06 ng/L		-	[37]

Australia	Biological WWT including constructed wetlands	Municipal wastewater	60 ng/L DEX equiv. (SBR) 51–85 ng/LDEX equiv.	Sequence batch reactor (SBR) below LQL Increases after chlorination 70 ng/L DEX equiv.		[23]
China	Biological Wastewater Treatment	Municipal wastewater	0.81 ng/L	0.03 ng/L	<0.02 ng/g	[38]

*Conc.: concentration; LQL: lower quantification limit

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