Studies on plastic derived residues in milk of cattle suffering from plastic impaction

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Abstract

Ruminal impaction with plastics is one of the common ruminal disorders in bovine especially in cities like Bengaluru that occurs due to scanty grazing areas, indiscriminate feeding habit of bovines and most importantly easy access to plastic covers. Six cows suffering from severe impaction containing heavy quantities of plastics were selected for the study. Standing left flank laparo-rumenotomy was performed under paravertebral nerve block and retrieved different quantities of indigestible foreign bodies including plastic bags, tubes, wires, threads, ropes, bone pieces, coins, sand *etc*. About 250 ml of milk was collected pre and 10 days post-surgery from six plastic impacted animals and analyzed for Polychlorinated biphenyles (PCBs), Dioxins and Phthalates by using GC-MS/MS. No PCBs and dioxins could be detected in the milk samples. Only four out of five phthalate congeners (DIBP, DEHP, DBP, BBP and DMP) (LOD-1.5 pg/g fat, LOQ-0.5 pg/g milk fat) were identified and quantified. Very high concentrations of phthalates before surgery in six animals were identified which ranged from 57.99-134.20 µg/g milk fat and were non-significantly declined after 10 days of post-surgery (ranged from 40.45 to 125.75 µg/g milk fat). These levels are also alarmingly high as per prevailing standards.

Key words: Cattle; plastic impaction; plastic derived residues; milk; GC-MS.

Introduction

Plastic is now-a-days ubiquitous, most widely used item and became almost undivided part of human life. The production of total plastics in India scaling up alarmingly, of which, major share belongs to single use plastic (wrappings, plastic bags, *etc*.) made of low density polyethylene that is posing major threat to both human and animal life currently (Wright and Kelly, 2017). At homes, usually the unwanted or left over foods will be packed in plastic bags or covers and thrown on to roads. These plastic bags at next level reach the dust bins and then dumping yards, where many stray or domestic cattle or buffaloes especially in urban areas ingest these indiscriminately in a confusion and due to their inability to separate edible material from inedible (Boodur, 2008) besides inhaling the toxins from burnt plastic at dumping yards.

Polychlorinated biphenyles (PCBs) are chlorinated hydrocarbons that are thermally stable with low electrical conductivity. Hence, they have been used in heat exchange fluids in transformers and capacitors, plasticizers, retarders, softeners, and lubricators (Cajal, 2007). There are 209 possible congeners of PCBs of which, 130 were found in the commercial mixtures that can be released to the environment. They cause wide variety of health hazards like skin rashes, eye irritation, disturbances in liver and immune system, and chronic effects like liver damage, cancer, and problems in human and animals' reproductive system. PCBs were also estimated in cow's milk all over the world in human health aspect (Focant *et al*., 2003; Vanitha *et al*., 2010). In poultry there are evidence of presence of metabolite melamine in eggs of laying hens following consumption of cyromazine contaminated feed (Bao et al., 2011).

Dioxins are a group of hazardous toxic chemicals that have an ability to enter environment or body and accumulate in fat tissues. There are about 75 types of dioxins, but, the most common and important one is 2,3,7,8 – Tetrachloro dibenzo-p-dioxin or TCDD which was designated as "human carcinogen". They are released in very minute quantities from chlorine containing materials like plastics, pesticide treated wastes, cigarette smoke, exhaust from vehicles, *etc*. (EPA, 2000) on burning, leaching and exposing to other environmental extremes. Milk and dairy products contribute major sources of dioxins and can stay for about 7–12 years in animal or human bodies (ATSDR, 2024). They were found to cause cancer, disrupt endocrine and reproductive systems and lead to developmental defects. Their presence was found in alarming quantities in cow milk all over the world (Focant *et al*., 2003; Someya *et al*., 2010; Bertocchi *et al*., 2015).

Phthalates are mainly used as plasticizers, *i.e*. substances added to plastics to increase their flexibility, transparency, durability and longevity mainly in PVC industry. Apart from PVC, phthalates are also been used in variety of products like enteric coatings of pharmaceutical pills, pesticides, detergents, adhesives, glues, packaging, soft toys, food containers and wrappers, blood transfusion sets, catheters, electrical insulations, *etc*. (Lorz *et al*., 2007). Di (2-ethylhexyl) phthalate (DEHP) is one of the compounds among the plasticizers used in plastic manufacturing that has been described by United States Environmental Protection Agency as a probable human carcinogen (causing lung cancer, asthma), a potential endocrine disruptor (reduce male fertility, obesity) and is believed to be harmful by inhalation, generating possible health risks and irreversible effects released during combustion of plastics (Verma *et al*., 2016). Many researchers quantified these toxins in cow milk throughout the world, but, no such comprehensive work in India was done to analyse for these three toxic compounds in milk collected from cattle suffering from severe and chronic plastic impaction.

Materials and Methods

The present work was carried out among six cattle presented for rumenotomy and retrieved with high quantity of plastics (more than 20 kg). From six selected animals, 250 ml each of fresh milk was collected before surgery and on 10^{th} day post surgery in stainless steel containers and stored in -20^0 C refrigerator till analysis. The analysis of milk for plastic derived residues was carried out at Sriram Institute for Industrial research, Bengaluru. Before analysis, the milk sample was thawed and 100 mg milk fat was separated.

Method of analysis for Polychlorinated Biphenyles (PCBs)

Around 100 mg of fat was weighed in a 250 ml beaker and 25 g of florisil was slowly added. Then 5 g sodium sulphate was added to the above mixture, gently stir it and ground the contents in a mortar and pestle. The column was packed with cotton that was washed previously with petroleum ether. 25 g of fresh florisil was filled in to the column. It was gently tapped to get a tight bed, and then pour 50 ml of elution solvent to it, so that the florisil will get well packed without any air gap. Now florisil coated with sample was loaded on top of the fresh florisil which was previously packed. The column was eluted with petroleum ether and dichloromethane mix (7:3). 300 ml of elute was collected and evaporated to nearly dryness but not completely dry. The sample was dried again after adding 1 ml n-Hexane. Then added another 2 ml of n-Hexane and transferred in to vial and injected to GC-MS.

Method of analysis for Dioxins

Around 100 mg of milk fat was weighed and dissolved with 5 ml of toluene and was passed through a set of disposable columns; a high capacity acid silica column, a small multi-layer silica column, a basic alumina column and activated carbon and elute with 100 ml of toluene, the eluent was evaporated under vacuum evaporator. The resulting solution was made up to 2 ml of toluene and injected to GC-MS/MS.

Method of analysis for Phthalates

Around 100 mg of fat was collected and weighed in a 250 ml beaker and dissolved and extracted with dichloromethane. Dichloromethane was collected and evaporated to dryness. 2 ml of n-Hexane was added and transferred in to vial and injected to GC-MS.

Estimation of plastic derived residues

Estimation of plastic derived residues was carried out using Gas Chromatography/ Mass Spectroscopy. Quantified Dioxins and PCBs with Agilent 6890 series equipped with electron capture detector and an automatic injector. Shimadzu TQ 8030 GC-MS/MS – was employed for quantifying Phthalates.

Results and Discussion

Collection of milk

Milk was collected in stainless steel containers only to avoid spoilage and chance of leaching of its contents (especially phthalates) in to milk which could alter the actual values (Fierens *et al*., 2013). Lin *et al*. (2015) collected milk in plastic containers and found a 10 fold increase in DEHP levels.

Estimation of plastic derived residues

Estimation of plastic derived residues in all the processed samples was done using gas chromatography/ mass spectroscopy. Agilent 6890 series equipped with electron capture detector (Vanitha *et al*., 2010; Ahmadkhaniha *et al*., 2017) and an automatic injector was used to quantify Phthalates and Shimadzu TQ 8030 GC-MS/MS was employed for quantifying Dioxins and PCBs. Perez *et al*. (2013) used high resolution GC; Fierens *et al*. (2013) used GC wt low resolution MS wt impact ionization detector and Chen *et al*. (2017) used GC-MS/ QuEChERS method for estimation of plastic derived residues in milk.

Polychlorinated Biphenyles

No PCB congeners (PCB 1016 and PCB 1245) could be identified (LOD-1.5 pg/ g fat and LOQ-0.5 pg/ g fat) in the milk samples collected on day 0^{th} and day 10^{th}. Shankar (2015) analyzed milk samples for the presence of 15 PCB congeners on day 0 and day 12 and could only identify them qualitatively but could not quantify them. Non-detection of PCBs in present study could be ascribed to absence or presence in low concentrations of these compounds at identifiable levels in polythene bags and Someya *et al*., 2010). The sample processing or method analysis also might be different from the studies in which the above pollutants indented. Vanitha *et al*. (2010) recorded high concentrations of PCB 146 and 155 of 5.32 and 1.54, 6.18 and 2.2 ppm in rumen liquor and milk respectively, but expressed failure of estimating in about 70 % samples by that method. Shankar (2015) did qualitative analysis and detected 15 PCB congeners in milk collected from plastic impaction cows. Except these two works, there was no much data available at disposal to compare or interpret our results.

Dioxins

No dioxins (TCDD) were detected and quantified (LOD-1.5 pg/ g fat and LOQ-0.5 pg/ g fat) in the milk samples collected before and on 10^{th} day of surgery and the reasons might be similar to that of PCB unavailability. Van Leeuwen *et al*. (2000) established a total daily intake range of 1-4 pg/ TEQ/ kg BW as indicated by WHO – ECEH (European Centre of Environment and Health). In other studies, milk had been analyzed and estimated to have PCDD concentrations of 1.26-13.01 pg/g fat (Focant *et al*., 2003), 0.40-13.0 pg/g fat (Someya *et al*., 2010) and 0.51-4.64 pg/g fat (Piskorska-Pliszezyrska *et al*., 2012).

Phthalates

Five Phthalate congeners, *viz*., Di-isobutyl phthalate (DIBP), Di-ethyl hexyl phthalate (DEHP), Di-butyl phthalate (DBP), Benzyl butyl phthalate (BBP) and Di-methyl phthalate (DMP) were estimated by GC-MS/MS technique as per availability of the standards. Except DIBP and DMP, other three could be identified and quantified (Table 1, Fig. 1, 2 and 3) in the milk samples. Very high levels of phthalates before (DEHP-18.45±3.16; DBP-25.76±9.92 and BBP-1.02± 0.41 µg/g fat) and after surgery (DEHP-15.03±2.81; DBP-22.15±8.75 and BBP-0.62±0.24 µg/g fat) were found in our study. The mean phthalate values showed non-significant decline but, still persisted in milk even after surgery by day 10^{th}. The quantity of plastics in rumens does not seem to affect the

Table 2: Overview of Phthalate concentrations (µg/g milk fat) in milk of bovines diagnosed with plastic impaction before and after surgery

		DIBP		DEHP		DBP		BBP		DMP	
Animal No.	**Quantity of plastics (kg)**	**Before surgery**	**After surgery**	**Before surgery**	**After surgery**	**Before surgery**	**After surgery**	**Before surgery**	**After surgery**	**Before surgery**	**After surgery**
1	33	0	0	22.30	12.60	0	0	0	0	0	0
2	32	0	0	11.30	9.02	0	0	0	0	0	0
3	23	0	0	11.40	10.90	22.50	18.80	0.99	0.69	0	0
4	53	0	0	30.30	26.30	58.90	52.10	1.08	0.72	0	0
5	55	0	0	22.40	20.70	48.50	42.20	1.30	0.74	0	0
6	57	0	0	13.00	10.70	24.70	19.80	2.77	1.58	0	0
Mean ±SE		**0**	**0**	**18.45 ± 3.17[a]**	**15.03 ± 2.81[b]**	**25.76 ± 9.92[a]**	**22.15 ± 8.75[b]**	**1.02 ± 0.41[a]**	**0.62 ± 0.24[b]**	**0**	**0**

Values with different superscripts vary significantly at p<0.01

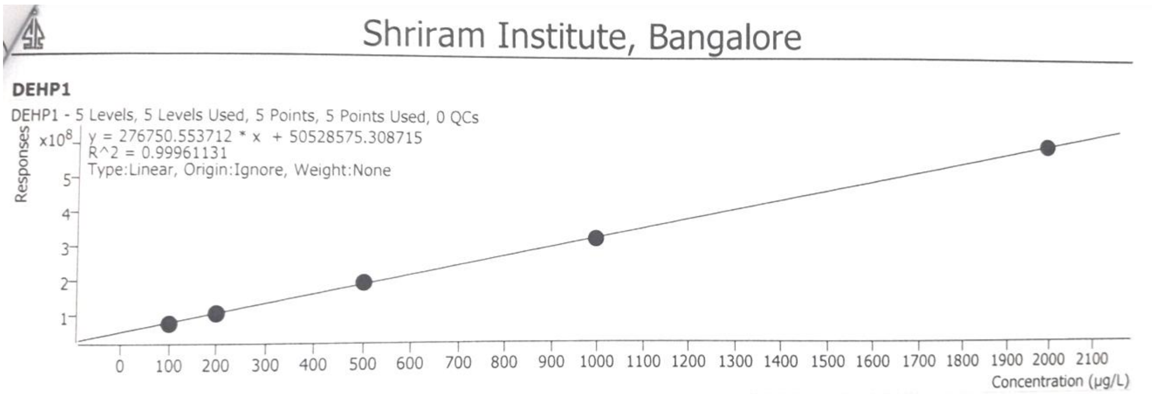
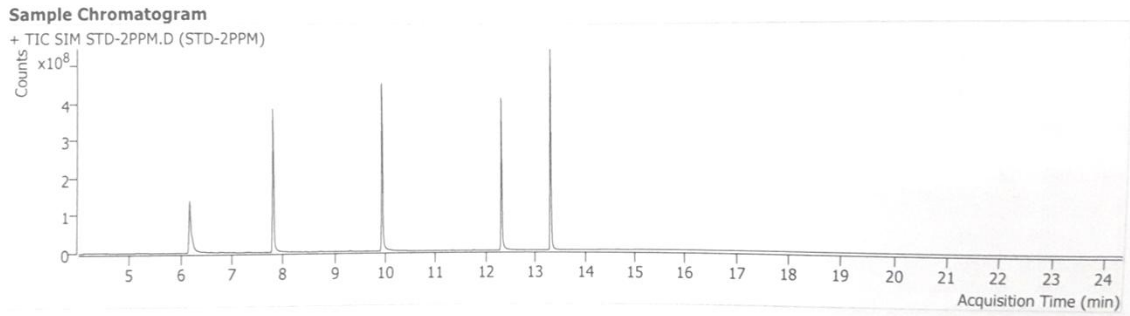


Fig. 1: Standard curve of Phthalates



Name	RT	Resp.	Final Conc. Units	Ratio
DMP	6.176	338300436	2001.1415 µg/L	11.1
DIP	7.795	334723574	2005.8243 µg/L	0.1
DBP	9.929	52196221	1998.3617 µg/L	51.2
BBP	12.315	136830131	1988.9126 µg/L	34.6
DEHP1	13.309	603210724	1997.0408 µg/L	3.3

Fig. 2: Standard Chromatogram of phthalates

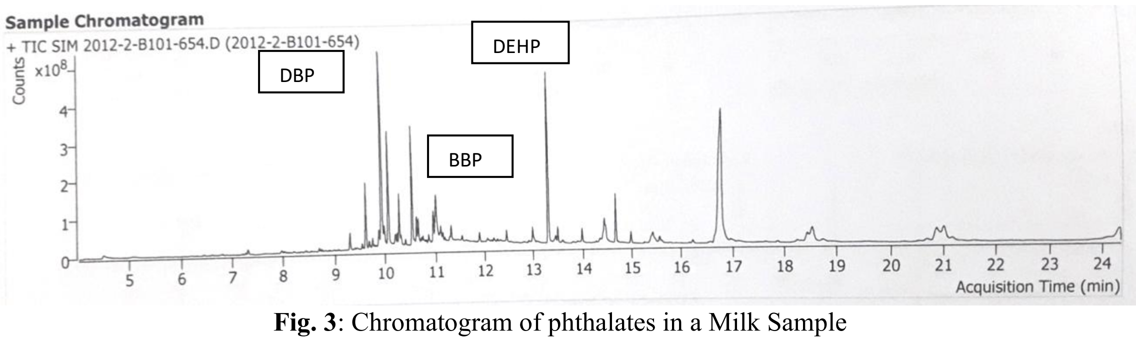


Fig. 3: Chromatogram of phthalates in a Milk Sample

concentrations of phthalates in milk. DEHP was present in highest quantities followed by DBP, DIBT and BBT at both intervals. These levels were very high compared to tolerable daily intake (TDI) value of 0.05 mg/ kg BW indicated by European Union for Scientific Committee for Food. Contrary to this, low concentrations of DEHP were quantified by various authors like Fierens *et al*. (2013) (0.356-0.478 µg/ g fat in fresh and processed milk), Serrano *et al*. (2014) (0.0084-0.117 and 0.139-2.27 µg/ g fat respectively in milk and cream), Senlik *et al*. (2014) (0.41-4.0 and 30.0-62.6 ng/ kg fat in fresh and packed milk respectively).

High lipophilicity is another important nature of the phthalates (Saad *et al*., 2015) and hence, they tend to accumulate in fatty tissues of body particularly in milk (Cao, 2010). Further, their concentrations were found to be exponentially increased in its concentrated products like creams, butter, cheese, *etc*. (Fierens *et al*., 2013; Senlik *et al*., 2014; Saad *et al*., 2015). There were no established standard threshold values of phthalates in India, but, compared to earlier studies at other places, estimated phthalates in pasteurized milk collected from super markets or dairy farms (Fierens *et al*., 2013; Lin *et al*., 2015), the concentrations of phthalates was higher in our study. This might be due to heavy leaching from plastics inside rumen and consequent accumulation into milk. Usually, the values must have become undetectable, once the source of phthalates was removed (Bertocchi *et al*., 2015) (removal of plastics by rumenotomy), but still higher levels were observed post operatively also. This could be ascribed to the mobilization of fat (during stress periods and for gluconeogenesis) from other sources in to milk.

Conclusion

The high levels of phthalates could be identified by GC-MS/MS. The high levels were still persisting in alarming levels even after complete elimination of plastics from rumen. Hence, a comprehensive study must be undertaken to standardize the normal levels of phthalates in milk and be compared with that of plastic impaction cases. Further, a threshold level of PCBs, dioxins and phthalates must be established in Indian sub continent. Studies have also to be conducted to know the reason why the phthalate levels are still persisting in blood and milk even after removal of the plastics.

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