Toxicological Evaluation of Milk from Lactating Dairy Cows following Consumption of Newsprint

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One of the uses that has been suggested for the extremely large waste stream of newspapers is for cattle bedding. Cows, however, are inclined to consume newspaper, which may contain a number of potentially toxic compounds including dioxins in the paper and benzidines in the inks used in the printing process. Conceivably, these compounds, many of which are lipophilic, could bioaccumulate in the milk of cows and thus enter the human food chain. In this study milk was collected from lactating cows before and after consuming a diet containing 10% newspaper for 2 weeks. The presence of mutagens was evaluated in the paper, diet, inks, and milk, and the concentration of dioxins was measured in the paper. Mutagens were found in newsprint, in the inks, and in two samples of milk. The concentration of the most toxic dioxin, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, in the paper was below the level of detection (0.5 ppt) but the concentration of hepta- and octachlorodibenzo-*p*-dioxin and heptachlorodibenzofurans ranged from 5.4 to 350 ppt. Although the results of this study suggest that the risk to human health from drinking milk from cows that consumed newsprint is less than that from drinking coffee or tea, caution is urged in the use of newsprint as cattle bedding material because of the variability and lack of regulation of constituents in newspaper and associated shredded waste.

INTRODUCTION

Waste newsprint comprises about half of the nation's solid waste stream (Haynes et al., 1977). Since only a relatively small percentage is recycled, a proposal has been made that disposal of a large part of the remainder could be through its use as animal bedding. Although cows voluntarily consume newsprint, the amount consumed is dependent on the ration offered to the animals (Grieve et al., 1982); the higher the concentration of hay in the ration, the lower the intake of newspaper. A concern, however, is that any toxic or mutagenic compound found in the newsprint that is consumed, particularly if it is lipophilic, could be secreted into the milk and subsequently consumed by humans.

Newsprint contains a galaxy of chemical compounds including whiteners and fillers which are added in the manufacturing process, dioxins which can be formed in the bleaching process, naphthenic oils used to solubilize the inks, and the inks themselves. Organic solvents including methylene chloride, acetone, ethylbenzene, xylene, toluene, and phenols have also been identified in newsprint (Sussman, 1989). Naphthenic oils, which are products of petroleum distillation, are refined by hydrogenation to remove sulfur and nitrogen compounds and then solvent-extracted to remove polycyclic aromatic compounds. The purified naphthenic oil consists mainly of fat-soluble saturated cycloparaffinic and isoparaffinic compounds which, if consumed, could conceivably be stored in fat depots or secreted into milk either unchanged or as a metabolite. Recently it has been shown that untreated or acid-treated naphthenic oils are mutagenic (Granella and Clonfero, 1991), possibly due in part to the presence of three- to seven-ringed polycyclic aromatic hydrocarbons (Roy et al., 1988). Due to the mutagenicity and bioaccumulation potential of these naphthenic oils, there has been a movement to replace them with soybean oil or water-based carriers, but at present only 400 newspapers use soybean oil and 60 use water-based carriers of a total of 16 000 daily and 7600 weekly newspapers in the nation (G. R. Cashau, 1989, American Newspaper Publishers Association, personal communication).

There are hundreds of different ink formulations used by ink manufacturers, and no legal restrictions exist as to which ones can be used on newsprint (*Thomas Register of American Manufacturers*, 1983). The rotogravure inserts in newspapers consist of colored illustrations which contain a number of pigments comprised of heavy metals such as lead in yellow lead chromate. Since this glossy paper absorbs moisture poorly, attempts are made to remove it from newspaper prior to chopping for animal bedding. Aside from these glossy rotogravure magazine sections of newspapers, the black and white portions also contain colored inks comprised largely of organic diazo compounds, many of which are mutagens (Chung and Cerniglia, 1992) and also carcinogens (Combes and Haveland-Smith, 1982). Mutagenic dyes have been divided into three groups depending on whether they contain nitro, phenylenediamine, or benzidine groups in the molecule (Chung and Cerniglia, 1992). Those dyes containing a nitro group are frequently direct-acting mutagens, whereas those containing phenylenediamine or benzidine moieties are promutagens. The phenylenediamine or benzidine moiety can be cleaved from the dye molecule either by azo reductases present in the microsomal fraction of a cell

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Table I. Composition of the Pelleted Diet

constituent	wt %	constituent	wt %
paper[a]	10.00	molasses	5.00
corn meal	43.95	minerals	1.00
alfalfa meal	25.00	vitamins	0.05
soybean meal	15.00		

[a] With or without ink.

Table II. Proximate Analysis of the Pelleted Diet

diet	protein, %	fat, %	ash, %	energy, kcal/g
10% paper without ink	15.53	2.90	6.92	4.381
10% paper with ink	16.26	2.74	6.78	4.427

preparation or following incubation of the dye with an intestinal bacteria under anerobic conditions (Chung, 1983). In the highly anaerobic environment of the rumen of a cow, it is likely that these mutagenic compounds could be cleaved from the azo dye following consumption.

The third group of toxic compounds which are found in newspaper are the polychlorinated dibenzo-*p*-dioxins (PCDD). A previous study showed that when PCDD was included in the ration of beef cattle at 24 ppt of 2,3,7,8-tetrachloro-*p*-dibenzodioxin (TCDD), it bioaccumulated in the edible tissue (Jensen et al., 1981). Also, if either radiolabeled TCDD (Jones et al., 1987) or 2,4,5-trichlorophenoxyacetic acid contaminated with TCDD was included in the ration of dairy cows or goats, TCDD was found in the milk (Arstila et al., 1981; Jensen and Hummel, 1982). The concentration of TCDD in the milk and its half-life were dependent on the concentration in the ration and the period for which it was fed. Heavy metals and polychlorinated biphenyls (PCB), which were used widely in newsprint a few years ago and are used to a limited extent today (Rastogi, 1992), have also been shown to be secreted into the milk of ruminants (Furr et al., 1974; Grieve et al., 1982) and to accumulate in tissues following ingestion of newspaper (Heffron et al., 1977). An analytical study of the presence of the myriad of compounds in the carrier oils, pigments, and newsprint would present an overwhelming challenge as the chemical composition of these mixtures is extremely variable. Thus, a more holistic approach to determine whether these compounds could partition into milk and thus pose a risk to man seemed to be more appropriate for this study. Since many of the synthetic compounds in the inks and naphthenic oils could potentially be mutagenic and a high percentage of mutagens are carcinogens (McCann et al., 1975), the Ames mutagenicity assay was used to analyze the paper, feed, inks, and milk samples for mutagens. To investigate whether dioxins, which are known contaminants of paper, were present in this newsprint, the concentration of these compounds in the paper fed to the cows was measured. In addition, the levels of PCB, chromium, and lead were also measured.

MATERIALS AND METHODS

Cattle Feeding. In April 1989, 50 lb (22.73 kg) of uncirculated newsprint and its respective virgin paper without ink (control) were obtained from two newspapers, the *Ithaca Journal* and *USA Today*. Care was taken to remove all rotogravure magazine sections and any soiled paper. The paper was shredded and then milled to a powdery consistency to pass through a stainless steel screen with 1 mm diameter openings. The milled paper, 10% (w/w), was mixed with the feed to yield a complete dairy ration and then made into pellets $^3/_{16}$ in. in diameter. The composition of the pelleted feed is listed in Table I and its proximate analysis in Table II.

Four Holstein cows were used in the feeding trial; two were assigned to each of the diets, one of which contained paper with ink and the second paper without ink. Data relating to the age, weight, pregnancy status, and milk production are shown in Table III. Each cow was fed 16 lb (7.3 kg)/day of the respective pelleted diet for 16 days with hay and corn silage offered ad libitum. Subsamples of the entire mixed afternoon collection of milk were obtained from each cow on the day before feeding of the paper rations began (absolute controls) and again on the 16th day after the feeding regimen began. The milk samples were divided in two, half was frozen immediately and stored at −18 °C and half was freeze-dried.

Table III. Data Pertinent to the Cows and the Corresponding Milk Production

dietary treatment	cow	cow age, years	cow wt, kg	days pregnant	milk production, kg/day: day before feeding paper	milk production, kg/day: last day of feeding paper
paper without ink	7	7.16	613.6	131	11.1	13.9
	17	4.42	518.2	92	15.0	17.3
paper with ink	59	5.75	590.9	np[a]	18.0	25.5
	88	4.58	486.6	np[a]	19.1	25.9

[a] Not pregnant.

Preparation of Samples for Testing in the Ames Mutagenicity Assay. Two extraction methods were used to prepare the samples for the Ames mutagenicity assay: a simple extraction with dichloromethane (DCM) for the paper and diets and a more complex fractionation procedure for the dyes and milk samples.

Extraction with DCM. Two powdered newspaper samples (20 g), one without ink and one with ink, and 25 g of the entire ration containing 10% (w/w) of paper with and without ink were extracted with 250 mL of DCM in a Soxhlet apparatus for 8 h. The extracts were evaporated to dryness under nitrogen and then resuspended in 1 mL of DMSO. A similar extraction procedure was used for the freeze-dried milk samples, but due to the high fat content, the samples could not easily be dissolved in small volumes of DMSO. Thus, a more complex method of fractionation of the inks and freeze-dried milk samples was used.

Fractionation of Milk Samples. A 0.5-g aliquot of the freeze-dried milk was added to 2.0 g of solvent-washed C_{18} (octadecylsilane-coated silica beads 40 μm in diameter) obtained from Analytichem, Harbor City, CA, as described by Barker et al. (1989) and Long et al. (1990). The sample was blended gently for about 30 s to obtain a homogeneous mixture using a glass pestle in a glass mortar. The mixture was poured into a glass syringe barrel containing a filter paper plug in the bottom. A small wad of glass wool followed by filter paper was placed on top of the column. Using gentle pressure of the syringe plunger, the column was carefully compacted to a height of approximately 4.5 cm. A pipet tip was placed on the bottom of the barrel to impede the flow rate of fluid through the syringe. The syringe was eluted sequentially with 8.0 mL of the following solvents: hexane, benzene, DCM, ethyl acetate, and methanol. Fractions of each solvent were collected and evaporated to dryness and then reconstituted in 1.0 mL of DMSO. The ink samples, which consisted of a number of components, were also fractionated and tested in the Ames assay.

Ames Mutagenicity Assay. The plate incorporation method of the Ames mutagenicity assay as described by Maron and Ames (1983) was used in the study. *Salmonella typhimurium* TA 98 and TA 100 were kindly supplied by Dr. Bruce Ames, Department of Biochemistry, University of California, Berkeley, CA. A 100-μL aliquot of a bacterial suspension from an overnight culture of the bacteria in Oxoid broth and a 50- or 100-μL aliquot of a DMSO extract of paper, ink, or milk was added to a tube containing 2.0 mL of top agar fortified with 0.05 mM histidine–biotin. For the determination of direct-acting mutagens, a 0.5-mL aliquot of 0.1 M phosphate buffer, pH 7.4, was added to the tubes, while for promutagens a 0.5-mL aliquot of an S9 mix was added. The S9 mix contained 8 mM $MgCl_2$, 33 mM KCl, 5 mM glucose 6-phosphate, 4 mM $NADP^+$, and an aliquot equivalent to 0.5 mg of protein/plate of a 9000*g* supernatant (S9) fraction. The S9 fraction was prepared from the liver of a rat pretreated

with Aroclor 1254 to induce cytochrome P-450 enzymes. The contents of the tubes were mixed and poured onto plates containing bottom agar fortified with 2% glucose and Vogel-Bonner salts. The plates were incubated for 48 h at 37 °C and the revertants enumerated using a Biotran colony counter. Negative controls contained DMSO and positive controls contained 5.0 μg of 2-nitrofluorene (TA 98) or 10.0 μg of sodium azide (TA 100) without S9 and 10.0 μg of 2-aminofluorene for both strains in the presence of S9. The extract was considered mutagenic if the number of revertants was twice that found in the negative control.

Analysis of the Paper Samples for Chlorinated Dibenzo-*p*-dioxins and Dibenzofurans. A 30-g aliquot of each milled paper sample, with and without ink, was extracted in a Soxhlet apparatus. Prior to extraction, an isotopically labeled standard mixture of $^{13}C_{12}$-labeled compounds 2,3,7,8-TCDD, 2,3,7,8-TCDF, 1,2,3,7,8-P_5CDD, 1,2,3,7,8,9-H_6CDD, 1,2,3,4,7,8-H_6CDF, and OCDD (Cambridge Isotope Laboratories, Woburn, MA) was added to each sample. Each thimble was then extracted for 24 h with 600 mL of DCM. After extraction, the samples were evaporated to 0.5 mL. A reagent blank was extracted in a similar fashion. The samples were subjected to a cleanup procedure involving an acid/base wash and multiple-column chromatographic cleanup steps (Tong et al., 1989b). The cleaned extracts were analyzed by high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS) using a Carlo-Erba GC/Kratos MS-50 mass spectrometer system. A 60-m DB-5 column (J&W Scientific, Folsom, CA) was used for the GC separation. The mass spectrometer was operated under electron impact ionization (70 eV) at 10 000 mass resolving power in the mass profile (MP) mode. Quantification was by the internal standard ratio method. TCDD and TCDF were quantified using [$^{13}C_{12}$]-2,3,7,8-TCDD and [$^{13}C_{12}$]-2,3,7,8-TCDF standards, respectively. P_5CDD and P_5CDF were quantified using [$^{13}C_{12}$]-1,2,3,7,8-P_5CDD. The $^{13}C_{12}$-labeled standards of 1,2,3,7,8,9-H_6CDD and 1,2,3,4,7,8-H_6CDF were used to determine H_6CDD and H_6CDF, respectively. The concentrations of H_7CDD and H_7CDF, OCDD, and OCDF were determined using [$^{13}C_{12}$]OCDD. The details of the HRGC/HRMS conditions, identification criteria, quantification by the internal standard ratio method, and mass profile monitoring were as described previously (Tong et al., 1989a, 1991).

Analysis of the Paper Samples for PCB, Chromium, and Lead. Total PCBs were extracted as Aroclor 1254 after Soxhlet extraction of the paper with hexane for 6 h. The U.S. Food and Drug Administration (1971) method (electron capture gas chromatography) was used to analyze for Aroclor 1254. The limit of detection of the method was 30 ppb. Chromium and lead were analyzed by emission spectrometry. Each paper sample (0.4 g) was dry ashed overnight at 450 °C. After the samples were cooled, 0.5 mL of 50% hydrogen peroxide was added and the mixture was evaporated to dryness in an oven (80 °C). The dry contents were again ashed at 450 °C for 2 h. The residue was dissolved in 1 mL of 12 N HCl using ultrasonic agitation for 1 h. The solution was diluted to 10 mL with distilled deionized water. Chromium and lead were determined using a Model 975 Jarrell Ash inductively coupled plasma emission spectrometer equipped with a fixed cross-flow nebulizer. The limits of detection for chromium and lead were, respectively, 50 and 350 ppb.

RESULTS

The cows consumed their daily ration of the paper-containing feed and showed no adverse effects based on an examination by a veterinarian. Both paper used in newsprint and newsprint itself were monitored for the presence of mutagens. A positive response both in the presence and in the absence of S9 was obtained with newsprint, with *S. typhimurium* TA 98 (Table IV) but not with TA 100 (data not shown). Paper without ink was negative with both bacterial strains. When paper with or without newsprint was added as 10% of the ration, however, no mutagenic response was elicited by the extracted feed sample (Table IV).

To determine which of the inks could be contributing to the response observed with the newsprint, extracts of four inks obtained from a local newspaper were evaluated for mutagenicity in *S. typhimurium* TA 98. With all of the inks, the fraction eluted in DCM was mutagenic in the presence of S9 and three inks, blue, red, and black, also contained direct-acting mutagens (Table V). In two cases (red and black inks), promutagens were present in the benzene fraction. The hexane fraction of the black ink also contained direct-acting mutagens.

Table IV. Mutagenicity of Paper and Feed Containing 10% (w/w) Paper in *S. typhimurium* TA 98

	no S9	with S9
paper with ink	74 ± 35[a]	140 ± 23[a]
paper without ink	35 ± 4	39 ± 14
feed + paper without ink	15 ± 5	14 ± 3
feed + paper with ink	17 ± 6	12 ± 3

[a] Twice background reversion rate.

Table V. Mutagenicity of Inks Used in Newsprint in *S. typhimurium* TA 98

	fraction	no S9	with S9
red ink	hexane	31 ± 13	19 ± 1
	benzene	39 ± 9	170 ± 130[a]
	DCM	45 ± 2	121 ± 36[a]
	ethyl acetate	21 ± 16	20 ± 1
	methanol	25 ± 8	16 ± 2
blue ink	hexane	18 ± 3	18 ± 1
	benzene	14 ± 5	23 ± 7
	DCM	110 ± 56[a]	184 ± 66[a]
	ethyl acetate	18 ± 2	29 ± 8
	methanol	21 ± 3	230 ± 2
yellow ink	hexane	14 ± 3	28 ± 5
	benzene	39 ± 5	41 ± 4
	DCM	483 ± 178[a]	429 ± 26[a]
	ethyl acetate	41 ± 8	37 ± 13
	methanol	11 ± 3	14 ± 2
black ink	hexane	191 ± 14[a]	279 ± 50[a]
	benzene	219 ± 34[a]	223 ± 47[a]
	DCM	258 ± 106[a]	401 ± 130[a]
	ethyl acetate	20 ± 7	29 ± 3
	methanol	51 ± 17	23 ± 8
negative control		19 ± 1[b]	20 ± 4[b]
positive control		348 ± 53[c]	1194 ± 171[c]

[a] Twice background reversion rate. [b] DMSO was used as the negative control in all experiments. [c] 5 μg of 1-nitrofluorene in the absence of S9 and 10 μg of 2-aminofluorene in the presence of S9 were used as the positive controls in all experiments.

It was interesting to note that promutagens were detected in the DCM extract of the milk sample collected at the end of the feeding regimen from cow 88, who received newsprint in her diet (Table VI). In addition, frameshift promutagens were detected in the benzene fraction of the milk from cow 59, who also received the diet with newsprint. No mutagens were detected in the fractionated milk from the cows that received paper without ink. The presence of base-pair mutagens in all of the fractions was evaluated in *S. typhimurium* TA 100, but no direct-acting mutagens or promutagens were observed (data not shown).

The newspaper used in these studies was analyzed for the presence of dioxins, one of the most toxic anthropogenic classes of compounds that has been detected in paper. To determine the concentration of these compounds, it is necessary to determine the percent recovery of each group of isomers. The respective percent recoveries of $^{13}C_{12}$-labeled TCDD, P_5CDD, H_6CDD, and OCDD in the blank were 110, 92, 68, and 67, in the DCM paper extract without ink were 84, 73, 83, and 94, and in the DCM paper extract with ink were 27, 38, 76, and 110. The concentration of TCDD, P_5CDD, H_6CDD, and P_5CDF was below the level of detection (0.3 ppt) in both the blank newspaper and

Table VI. Mutagenicity of Milk, after Fractionation, from Cows before and after Feeding 10% Newsprint in the Diet for 16 Days

		before newsprint feeding		after newsprint feeding	
cow	fraction	no S9	with S9	no S9	with S9
7[a]	1[b]	14 ± 3[c]	28 ± 5	38 ± 3	71 ± 11[d]
	2	15 ± 1	18 ± 3	19 ± 1	27 ± 5
	3	14 ± 3	17 ± 2	14 ± 4	20 ± 3
	4	23 ± 3	31 ± 2	35 ± 4	29 ± 7
	5	26 ± 3	32 ± 9	21 ± 1	29 ± 1
17	1	15 ± 3	15 ± 1	12 ± 4	17 ± 1
	2	15 ± 3	23 ± 5	18 ± 3	20 ± 4
	3	15 ± 2	21 ± 3	17 ± 4	16 ± 4
	4	27 ± 5	22 ± 2	26 ± 8	31 ± 4
	5	23 ± 3	13 ± 3	19 ± 2	24 ± 8
59	1	21 ± 1	28 ± 5	20 ± 6	27 ± 2
	2	15 ± 2	32 ± 7	18 ± 2	50 ± 10
	3	19 ± 4	37 ± 3	16 ± 2	24 ± 8
	4	18 ± 3	38 ± 7	20 ± 4	27 ± 4
	5	20 ± 7	26 ± 3	21 ± 4	28 ± 7
88	1	27 ± 18	75 ± 56	18 ± 3	36 ± 8
	2	22 ± 5	37 ± 18	25 ± 6	38 ± 18
	3	17 ± 8	60 ± 55	25 ± 2	65 ± 9[d]
	4	23 ± 1	284 ± 181	16 ± 3	37 ± 22
	5	25 ± 4	73 ± 51	20 ± 5	22 ± 6
negative control		13 ± 6[e]	29 ± 5[e]		
positive control		616 ± 8[f]	831 ± 50[f]		

[a] Cows 7 and 17 consumed the diets containing paper without ink, and cows 59 and 88 consumed diets containing paper with ink. [b] Fractions 1, 2, 3, 4, and 5 were eluted with hexane, benzene, dichloromethane, ethyl acetate, and methanol, respectively. [c] *S. typhimurium* TA 98 was used as the indicator organisms for these experiments. [d] Twice background reversion rate. [e] DMSO was used as the negative control in all experiments. [f] 5 μg of 1-nitrofluorene in the absence of S9 and 10 μg of 2-aminofluorene in the presence of S9 were used as the positive controls in all experiments.

that with newsprint (Table VII). However, detectable concentrations of H_7CDD, H_7CDF, and OCDD were found in both newspaper samples. Interestingly, the concentration of these dioxins was higher in the paper without ink than in the paper with ink. A detailed analysis of the individual hepta isomers is shown in Table VIII. The sum of the two major isomers 1,2,3,4,6,7,8- and 1,2,3,4,6,8,9-CDD and -CDF was essentially equal to the total concentration of the hepta isomers shown in Table VII. As the most toxic dioxin isomers, TCDD and the P_5CDD (Rappe et al., 1987), were below the level of detection (0.3 ppt) in the paper, it was deemed unnecessary to undertake the extremely expensive analysis of measuring the concentration of these dioxin and furan isomers in the milk of the cows.

PCBs were not detected in either paper sample. This is not surprising since the use of PCB as dye carriers in printing inks has been largely phased out. The respective concentrations (parts per million) of chromium and lead in the paper sample with ink were 0.97 and 0.39, whereas in the paper without ink chromium was present at 0.71 ppm but lead was below the level of detection of 0.35 ppm. Therefore, the contribution of the inking process in increasing the concentration of these metals in newsprint was minimal.

DISCUSSION

Mutagens were found in the inks, in the paper fed to the cows, and in two samples of milk from the cows receiving newsprint in their diets. The presence of mutagens in two milk samples suggests that mutagens were transferred through the food chain. Although all of the dyes were shown to be mutagenic, it is difficult to know which ones were secreted into the milk.

The chemical compositions of the dye pigments used by the local newspaper and supplied to us for analysis were unknown; however, the yellow pigment most frequently used for this application, yellow 12, is a benzidine-based azo dye (Legnetti, 1989, personal communication) which could be mutagenic in the presence of S9. In fact, we found that the yellow dye was both a direct-acting mutagen and a promutagen. The direct-acting response could have been due to the presence of the nitro-containing dye, arylide yellow, which is frequently used in newsprint (Legnetti, 1989, personal communication).

The red pigment used in the newsprint fed to the cows was found to be a promutagen (Table V). The most widely used red pigments for this application are lithol rubine and lithol red, which are similar in structure to Ponceau red 3R. This latter pigment was shown by Brown and Dietrich (1983) and Hartman et al. (1979) to be mutagenic in *S. typhimurium* TA 98 with S9 activation, probably due to its cleavage to 2,4,5-trimethylaniline, which is mutagenic in the Ames assay (Hartman et al., 1979).

Less is known about the constituents of the blue and black pigments. Although the most frequently used blue pigments are phthalocyanine blue and alkali blue, there is no information on the mutagenicity of these particular dyes. On the basis of the structure of phthalocyanine blue, it is possible that this molecule could be reduced to a phenyl diaminopyrazole intermediate, which could be a promutagen. The chemical structure of the black pigment (carbon black) is unknown, although carbon black, which is also used in photocopier toners, contains a number of direct-acting mutagens (nitro polycyclic aromatic hydrocarbons) and promutagens (benzo[*a*]pyrene) (Lofroth et al., 1980; Rosenkranz et al., 1980).

Although the total concentration of PCDD and PCDF obtained in our study was similar to that reported by Sussman (1989), the relative proportions of the various isomers differed, and thus the toxic equivalents of the two newspapers studied were also different. The predominant dioxin and furan isomers identified in the newspaper analyzed in the present study were H_7CDD, OCDD, and H_7CDF, while TCDD and P_5CDD were not detectable. In contrast, the concentrations of 2,3,7,8-TCDD and TCDF were much higher in the newspaper sample described by Sussman (1989). According to Rappe et al. (1987) and Safe (1986), the most toxic PCDD and PCDF isomers are 2,3,7,8-TCDD and TCDF, 1,2,3,7,8-P_5CDD, and three hexaisomers, namely 1,2,3,6,7,8-H_6CDD, 1,2,3,7,8,9-H_6-CDD, and 1,2,3,4,7,8-H_6CDD. None of these isomers was found in significant concentration in the newspaper used in our study.

In spite of the finding that PCDD and PCDF isomers were found in the newspaper fed to the cows in this study, we did not deem it essential that the concentration of these compounds be evaluated in the milk. This decision was made in light of the study of McLachlan et al. (1990) in which they found that only 25–35% of TCDD, P_5CDD, and P_5CDF, 15% of the hexa-substituted dioxins and furans, and 5% of the hepta- and octaisomers were secreted into the milk of cows fed an uncontaminated feed. Furthermore, the bioconcentration factor of TCDD was calculated to be 12.2 by both McLachlan et al. (1990) and Connett and Webster (1987), so that even if TCDD had bioaccumulated in the milk of the cows in our study, the concentration of this isomer would probably have been below the level of detection of 0.3 ppt. The data of McLachlan et al. (1990) indicate that those isomers with

Table VII. Concentration of PCDD and PCDF in Extracted Paper Samples

	pg/g of paper									
	TCDD[a]	P_5CDD	H_6CDD	H_7CDD	OCDD	TCDF	P_5CDF	H_6CDF	H_7CDF	OCDF
paper 1	ND[b]	ND	ND	62	150	1.9	ND	2.1	94	185[d]
extract (− ink)	(0.6)[c]	(1.1)	(1.1)	(1.1)	(1.6)	(0.3)	(0.6)	(0.5)	(1.0)	
duplicate	ND	ND	ND	66	150	3.1	ND	5.8	100	350
	(1.7)	(1.0)	(−)	(1.0)	(1.2)	(0.6)	(0.5)	(1.6)	(1.4)	(1.4)
paper 2	ND	ND	ND	5.4	43	ND	ND	0.8	37	110
extract (+ ink)	(2.2)	(1.4)	(1.4)	(1.0)	(−)	(0.3)	(0.4)	(0.5)	(1.4)	(−)
duplicate	ND	ND	ND	7.8	39	ND	ND	ND	36	100
	(−)	(2.6)	(0.9)	(0.8)	(1.5)	(3.0)	(0.6)	(1.7)	(1.1)	(1.7)
blank	ND	13	9.6	ND	24	2.5	ND	ND	ND	2.8
	(0.5)	(1.5)	(1.1)	(1.0)	(2.1)	(0.3)	(0.6)	(0.5)	(5.9)	(1.9)

[a] TCDD refers to tetrachlorodibenzo-*p*-dioxin; P_5CDF refers to pentachlorodibenzo-*p*-dioxin, etc. [b] ND indicates that the concentration was below the level of detection. [c] Detection limits in parts per trillion (ppt) are given in parentheses. [d] This value is not reliable as the detector was saturated.

Table VIII. Concentration (Parts per Trillion) of Individual Isomers of H_7CDD and H_7CDF in Paper Extracts

	H_7CDF		H_7CDD	
	1,2,3,4,6,7,8	1,2,3,4,6,8,9	1,2,3,4,6,7,8	1,2,3,4,6,7,9
paper extract (− ink)	47	57	61	5.0
paper extract (+ ink)	17	20	5.6	2.2

a high K_{ow} were less likely to be secreted into the milk and that these highly chlorinated isomers were absorbed to a lesser extent from the digestive tract than the lower chlorinated isomers. Their findings were corroborated by the study of Jones et al. (1987) in which they found that more than 80% of a radioactive dose of TCDD was excreted in the feces following its inclusion in the diet of a Holstein cow. Jensen and Hummel (1982) also showed that TCDD could not be detected in milk obtained from dairy cows receiving 5 ppt of TCDD in their diet, although measurable levels were found in the cream from the milk. Similarly, Riss et al. (1990) showed that anthropogenically produced PCDD and PCDF bioaccumulated in the cream fraction of milk from a cow living in an industrial area in which were located both incineration and chemical plants (Beck et al., 1990). Another pathway in which milk can become contaminated by PCDD and PCDF is through transfer of these compounds from cardboard containers when about 10% of TCDD equivalents can be leached into milk (Beck et al., 1990). In the United Kingdom the Department of Health has set the maximum tolerable concentration of dioxins in milk at 0.7 ppt on the basis of the World Health Organization European Region recommended tolerable daily intake of 0.01 mg/kg of body weight.

On the basis of our finding of the sporadic presence of mutagens in the milk (not detected in most cases) and the more than 100-fold concentration of the original milk sample that was required to obtain a response, it is concluded that the presence of mutagens in two of these samples should be considered in relation to other foods with regard to human health. Many other foods such as fried beef (Larsen et al., 1990) and beverages including coffee (Shane et al., 1988; Sugimura et al., 1986) and tea (Nagao et al., 1986) are more mutagenic on a unit weight or volume basis than the equivalent volume of milk obtained from the cows consuming small amounts of newspaper. Thus, we conclude from our findings that the risk to human health associated with drinking one glass of milk from the cows consuming newspaper bedding of the type fed in this study is lower than the risk associated with the consumption of an equivalent volume of many other beverages.

Among all of the dioxin and furan isomers examined, only the levels of the hepta- and octaisomers were above background levels in the paper. Even if these isomers had been secreted into the cow's milk, which seems unlikely on the basis of the earlier study of Jensen and Hummel (1982), the cumulative toxic equivalents of these isomers are 1/100 to 1/1000 lower than that of TCDD (Barnes et al., 1986). It has been estimated that the average daily intake of 2,3,7,8-TCDD in milk by humans is 0.013 ng/day, which is about 27% of TCDD consumed (Travis and Hattemer-Frey, 1987). As it is unlikely that TCDD or the P_5CDD isomers were secreted into the milk at detectable levels, we can conclude that milk from these cows is unlikely to contribute to the daily intake of TCDD by humans.

Although the less toxic dioxin isomers were detected in the paper and mutagens were detected in two milk samples, it is not possible to safely recommend the use of shredded newsprint for bedding for dairy cattle for a number of reasons. Among foods, milk receives the highest priority by regulatory agencies since it is commonly a major dietary constituent for infants, invalids, and the aged, i.e., the population groups most sensitive to toxicants. The major problem, however, is the variability in composition of waste paper. The multiplicity of chemical constituents that legally can be used in newsprint from the many commercial sources and our lack of knowledge of their identity, concentration, and toxicological properties (lipophilicity, metabolism, mutagenicity, and carcinogenicity) and the resultant uncertainty of appropriate analytical methods restrict scientists in making an accurate assessment of risk. Another deterrent is the failure of the recycling public and commercial operators of paper shredding equipment to remove rotogravure or soiled contaminated paper and packaging previously used for toxic chemicals from the waste stream. Indeed, the authors have personally observed the inclusion of a cardboard box that had contained rat poison with waste paper which was to be shredded by a commercial operator for dairy cattle bedding. Other concerns include our lack of knowledge of the fate of chemicals in newsprints in soil when this paper bedding and its associated manure are applied to land and plowed in or the unsightliness, especially in suburban areas, of wind-blown paper if this manure and bedding are applied to the surface. Other problems concerning the use of chopped paper for farm animals include the darkening of sheep's wool due to the absorption of dyes in print by lanolin in the wool and the slipperiness of cement floors in barns caused by paper, especially rotogravure print.

Perhaps the present increase in efforts to recycle paper into new products will reduce the current surplus and therefore diminish its use for cattle bedding. Farmers

who choose to use shredded newspaper for bedding should be informed of the importance of feeding a balanced diet with adequate roughage to discourage the consumption of newsprint by dairy cows.

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