Bull. Environ. Contam. Toxicol. (1986) 37:246–252

Environmental Contamination and Toxicology

Distribution of Indole in Tissues of Dairy Cattle, Swine, and Laying Pullets

G. R. Eisele

Medical and Health Sciences Division, Oak Ridge Associated Universities, P.O. Box 117, Oak Ridge, TN 37831-0117

Indole is a colorless crystalline solid which has been isolated from coal tar fractionation. This neutral (N,O,S,) heterocyclic compound is also found in animal tissues where putrefactive processes have occurred, presumably by the decomposition of tryptophan (Van Order and Lindwall, 1942). High concentrations of indole (which is a major ruminal fermentation product of L-tryptophan) in blood of cattle causes hemolysis, hemoglobinuria, and renal necrosis (Hammond et al., 1980). An end product of anaerobic metabolism of the colonic flora, indole has also been examined as a marker in patients with unresected large bowel cancer or polyps (Karlin et al., 1985).

With the increased release of numerous chemical substances into the biosphere, careful assessment of the health effects of chronic exposure to pollutants must be made. Many of these compounds are known toxic substances and/or are closely related chemically to recognized carcinogens and mutagens. The introduction of various chemicals into the food chain will be a major source of human risk. Much of the body burden of animals will come from ingested feed and water, with the primary route of human exposure being the consumption of the contaminated meat, milk, and eggs. Similar findings were reported for the diaromatic hydrocarbon naphthalene (Eisele, 1985). The purpose of this study was to obtain baseline data on the uptake and distribution of indole in dairy cattle, swine, and laying pullets and the retention of this chemical in consumable products such as milk, meat, and eggs.

MATERIALS AND METHODS

Laying pullets, swine, and dairy cattle received, via gavage, tracer levels of ^{14}C-indole in ethanol (specific activity 9.5 mCi/m mole, radiochemical purity 99%, ICN Pharmaceuticals, Inc.) either as an acute single or a chronic (30d) exposure. Pullets (four per time point) were individually housed and fed a complete laying ration and water ad libitum, and eggs were collected daily for analysis. The internal yolk sample was comprised of 6-8 of the largest yolk on the stalk. Swine (three per time point), weighing approximately 23 kg, were maintained in individual holding cages,

and the Holstein dairy cow was in an individual paddock area. The dairy cow was milked twice daily, and the ^{14}C-residue content of the milk was determined. The acutely dosed pigs and chickens were killed 1 or 3 days post exposure, and the chronically dosed animals were killed the day after the last exposure (day 31). One dairy cow was killed 3 days after the acute exposure and another 3 days after the last chronic exposure (34 days). The dairy cow, growing swine, and laying pullets received acute doses of 6100 uCi (75.23 mg), 400 uCi (4.93 mg), and 100 uCi (1.23 mg) of ^{14}C-indole, respectively.

In the chronic experiments, the dairy cow, swine, and pullets received 626 uCi/day (7.72 mg/day), 76 uCi/day (0.94 mg/day), and 38 uCi/day (0.47 mg/day), of ^{14}C-indole, respectively, for 30 consecutive days. At sacrifice, the following tissues were taken from all animals: two muscle samples, pelvic fat, and liver. The following muscles were sampled: M. peronaeus and M. pectoralis, referred to in the text as dark and white meat in pullets; and M. longissimus and M. biceps, referred to in the text as loin-and-ham in swine and loin and flank in dairy cattle. Triplicate samples of tissue, each approximately 0.1 g, were placed directly into standard scintillation vials to which 1 ml of tissue solubilizer (Unisol, Isolab, Inc., Akron, Ohio) was added. After solubilization, 0.5 ml methanol, 10 ml Unisol Complement (Isolab, Inc., Akron, Ohio) and 0.1 ml 30% hydrogen peroxide were added. Samples were counted for radioactivity in a Mark III liquid scintillation counter (Searle Analytic, Inc., Des Plaines, Illinois) which has a 96% efficiency for carbon-14 under these conditions. Appropriate standards were prepared and counted at the same time as the samples to correct for quenching and counter fluctuations.

RESULTS AND DISCUSSION

Results obtained with laying pullets following an acute and chronic exposure of ^{14}C-indole showed the major tissue sites of deposition were the internal yolk and liver, followed by muscle and fat which were approximately the same (Table 1). The internal yolk compartment consists of yolks in various stages of development which will be released from the stalk when mature for subsequent egg formation. The internal yolk values strongly suggest that even after 72 hours following an acute exposure (with slightly less than half the ^{14}C-activity removed in the previous 48 hours), eggs from these pullets still contained residue of this chemical or its metabolite. White and dark meat showed no significant reduction between 24 and 72 hours, suggesting a slow removal rate; yet, over 70% of the ^{14}C-indole was removed from body fat during this same time period. It is interesting to note that dark meat retained almost twice as much indole as did white meat.

The uptake of indole by eggs after acute exposure of laying pullets to indole is shown in Figure 1. The values were derived from two

Table 1. Distribution of indole or its metabolic products in tissues of laying pullets (fraction of dose/kg tissue 10^{-3})[a]

Tissue	Acute Dose 24 hours	Acute Dose 72 hours	Chronic Dose[b] 31 days
Liver	10.55 ± 0.98	3.14 ± 0.32	2.13 ± 0.26
Fat	1.80 ± 0.55	0.51 ± 0.08	0.33 ± 0.04
Dark Meat	2.89 ± 0.13	2.35 ± 0.25	0.88 ± 0.12
White Meat	1.82 ± 0.07	1.03 ± 0.07	0.78 ± 0.13
Internal Yolk	16.14 ± 2.55	7.58 ± 1.02	1.98 ± 0.11

a - mean ± SE, based on four animals
b - fraction of daily dose

Table 2. Distribution of indole or its metabolites in tissues of swine (fraction of dose/kg tissue 10^{-3})[a]

Tissue	Acute Dose 24 hours	Acute Dose 72 hours	Chronic Dose[b] 31 days
Liver	4.86 ± 0.60	2.66 ± 0.53	3.99 ± 0.41
Fat	0.76 ± 0.12	0.30 ± 0.07	0.42 ± 0.08
Loin	0.76 ± 0.04	0.68 ± 0.08	1.72 ± 0.32
Ham	0.65 ± 0.07	0.61 ± 0.14	1.79 ± 0.37

a - mean ± SE, based on three animals
b - fraction of daily dose

groups of laying pullets. The first group was killed 24 hours after exposure; the other values were derived from the group maintained through 72 hours. Within the first 24 hours, detectable amounts of indole were incorporated into eggs with subsequent egg formation showing continued excretion by this route. Between group (animal) variation is shown by one 24 hour group yielding values approximately twice that of the other 24 hour group (Figure 1). The internal egg (int. egg), which is an egg in some stage of development within the tract, still maintained this high level of incorporation past 72 hours.

The chronically exposed pullets (Figure 2) showed that within the first 24 hours post exposure, little indole was incorporated. This incorporation progressively increased up to approximately 16 days post exposure. After this time the values decline, possibly due to activation of the detoxifying processes of the body, and egg production was no longer the major excretory route. Within the first six days the ^{14}C-residue was about equally distributed between yolk and albumin in eggs from chronically exposed pullets. After this time, the predominant site of deposition was yolk. A similar distribution was implied in eggs from acutely

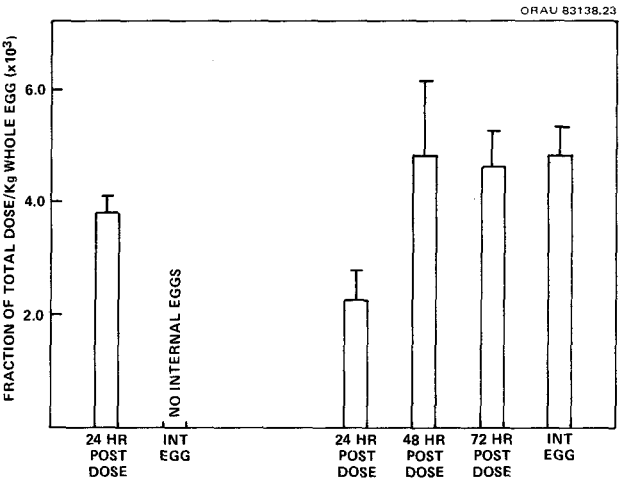

Figure 1. Fraction of dose of indole in eggs after an acute exposure. Each bar represents the mean ± standard error of four eggs.

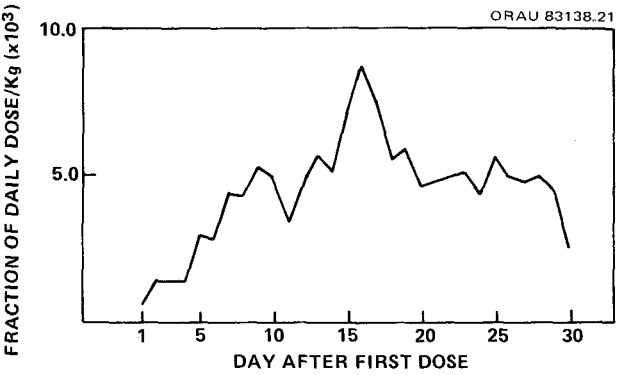

Figure 2. Fraction of dose of indole in eggs during a chronic exposure (30 days). Each day represents the mean of four eggs.

exposed pullets; equal distribution one and two days later, then five times more ^{14}C residue found in yolk on the third day. The body distribution in the chronic feeding experiment shows liver and internal yolk retaining approximately equal amounts of the ^{14}C-residue. In the acute study, the internal yolk was three to eight times higher in retention than in the chronic study (Table 1), which can be seen in the reduced values found in whole eggs (Figure 2). In both the acute and chronic studies, no adverse effects of ^{14}C-indole ingestion were noted on egg production, feed intake, or body weight.

Results of the acute and chronic exposure of swine to ^{14}C-indole are shown in Table 2. The major site of concentration 24 hours

after administration was the liver; at 72 hours after exposure this value had decreased by a factor of two. Fat and loin and ham muscles retained low levels of ^{14}C. The level in the fat was reduced by approximately one-half at 72 hours after administration, but there was little reduction in the level in muscle. The chronic exposure data show liver to be the major site of disposition followed by muscle. The level of ^{14}C residue in the muscle compartment of the chronic animals showed twice the retention than that observed in the acute animals, suggesting that the type of exposure (acute versus chronic) may have an important bearing on retention. Also, four times more ^{14}C residue was found in muscle than in fat.

Results of experiments with dairy cows exposed to an acute or chronic dose of ^{14}C-indole (Table 3) show that liver was the organ of major deposition with pelvic fat having the lowest. Although the tissue distribution was comparable in animals receiving both the acute and chronic exposures, animals given the acute exposure retained approximately two to three times the amount of radioactivity in the tissues. The fraction of the ^{14}C-indole administered acutely that was found in milk is shown in Figure 3. Within eight hours after exposure, ^{14}C-indole was detected in milk. A maximum value was reached at 24 hours with subsequent reductions by 72 hours. The distribution of ^{14}C-indole in milk during a 30-day exposure is shown in Figure 4. The chronic ingestion data suggest a biomagnification in milk. The dotted line from 30 to 33 days shows the elimination of ^{14}C-activity after the last administered dose. Although clearance from milk was evident during this three-day period, it did not decrease below the
first day's value, suggesting that a longer time period would be required to achieve nondetectable content in milk.

While this study provides specific data on the uptake and distribution of ^{14}C-indole, many other chemicals and their metabolites reach humans through the food chain. The primary route of exposure to food producing animals will be from ingested feed and water at relatively low exposure levels. Few observable manifestations, e.g. dysphagia, anorexia, dyspnea, will be evident, making an accurate evaluation difficult. Products such as milk and eggs are consumed directly and as add-in ingredients with a long shelf life, possibly extending the length of the insult.

The level of a chemical in the environment may only be an indicator of potentially higher levels in food-producing animals and subsequent food products. Biomagnification, the ability of animals to biochemically concentrate and accumulate chemicals in tissues, plus food producing procedures, may allow greater levels to concentrate in food products. The extent of contamination of man's food web is also dependent upon the species and the type of exposure. The differences between the fraction of the dose absorbed, acute versus chronic, indicates that the type of exposure is of primary importance in relation to retention. The

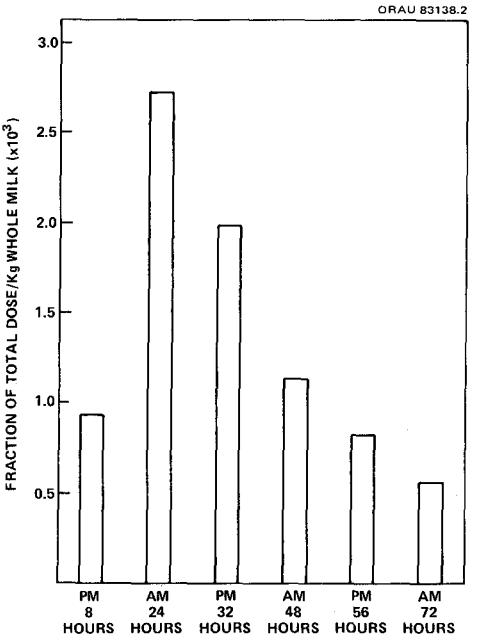

Figure 3. Fraction of dose of indole in milk of a dairy cow after an acute exposure. The bars represent the values from one dairy cow.

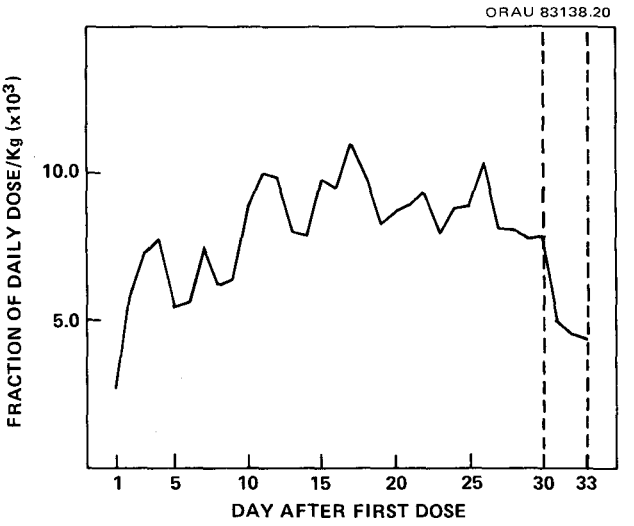

Figure 4. Fraction of dose of indole in milk of a dairy cow during a chronic exposure (30d). Each day represents the mean of the morning and evening milking.

average yearly consumption of eggs and poultry (ready to cook) is approximately 37 kg, which excludes many poultry by-products used as filler in other products. Milk is a major constituent in diets and is widely used as an add-in ingredient. Data from the

Table 3. Distribution of indole or its metabolites in tissues of dairy cattle (fraction of dose/kg 10^{-3})

Tissue	Acute Dose[a] 72 hours	Chronic Dose[a] 34 days
Liver	2.56	0.76
Fat	0.12	0.04
Loin	0.20	0.20
Flank	0.35	0.19

a - one sample.

chronically exposed dairy cow suggests a biomagnification occurring in milk, thus increasing exposure by this product. This was also observed in eggs. Both milk and eggs are used extensively as add-in ingredients in numerous products which have a long shelf life. If the exposure levels were higher, it is hypothesized that higher levels of indole or its metabolites would be found in the various tissues and products. These data suggest that the neutral (N,O,S,) heterocyclics, such as indole, can enter man's food chain. The extent of this insult appears to be small; however, little is known about the long-term consequences of such exposures at low levels over extended periods of time.

Acknowledgements. The author acknowledges the assistance of J. Payne, B. Kyle, and I. Urso. Although the research described in this article has been funded wholly or in part by the EPA through Interagency Agreement DW89930309-01-0 to the U.S. DOE, it has not been subjected to EPA review and does not necessarily reflect the views of EPA, and no official endorsement should be inferred.

REFERENCES

Eisele GR (1985) Naphthalene distribution in tissues of laying pullets, swine, and dairy cattle. Bull Environ Contam Toxicol 34:549-556.

Hammond AC, Carlson JR, Breeze RG (1980) Indole toxicity in cattle. Vet Rec 107:344-346.

Karlin DA, Mastromarino AJ, Jones RD, Stroehlein JR, Lorentz O (1985) Fecal skatole and indole and breath methane and hydrogen in patients with large bowel polyps or cancer. J Cancer Res Clin Oncol 109:135-141.

Van Order RB, Lindwall HG (1942) Indole Chemical Rev 30:69-96.

Received October 4, 1985; accepted October 20, 1985.