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

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Naphthalene is widely used in the manufacture of synthetic resins, dyes, etc., and is abundant in coal tar and synthetic and natural crude oils. Bock, Clausbruch, and Winne (1979) used isolated intestinal loops from rats to study the absorption and metabolism of naphthalene. They reported that naphthalene rapidly absorbed into the portal blood was mostly unchanged; the major ether-soluble metabolites were naphthalene-1,2,-dihydrodiol, and 1-naphthol. Van Heyningen (1979) concluded that cataracts were caused by the naphthalene metabolite 1,2-dihydroxynaphthalene in the rat and rabbit. Rao and Pandya (1981) reported that rats given naphthalene orally for 10 days showed a significant increase in liver weight, a two-fold increase in liver aniline hydroxylase, and no change in kidney weight. These findings are similar to those observed for animals exposed to insecticides, polycyclic aromatic hydrocarbons, and some drugs (Hermann, 1974). Horning et al. (1980) isolated 21 oxygenated metabolites of naphthalene from rat urine and found the anti-diepoxide compound formed from naphthalene metabolism was lethal in moderate dosages to rats and mice. The mechanism of epoxide toxicity is unknown.

The accumulation of azaarenes by a variety of marine life has been demonstrated, and the extent of this accumulation appears to increase on the basis of the number of aromatic rings in the compound (Southworth et al., 1978). There is additional supportive evidence that aromatic hydrocarbons (Ogata and Miyaki, 1973; Lee et al., 1972) and azaarenes (Southworth, 1979) can accumulate in tissues of fish and other marine animals and may be absorbed by humans when these tissues are eaten. Ekrhardt (1972), citing the data by Blumer (1967), concluded that higher-order animals can accumulate the entire range of hydrocarbons to which they are exposed through their food source. The purpose of the present studies was to obtain baseline data on the uptake and distribution of naphthalene in food-producing animals (laying pullets, swine, and dairy cattle) and the retention of this toxicant in consumable products (meat, eggs, and milk).

MATERIALS AND METHODS

Laying pullets, swine, and dairy cattle were given tracer levels of ^{14}C-naphthalene (specific activity 52 mCi/m mol, radiochemical

purity 97%, Amersham Corporation) orally, either as a single exposure and killed 1 or 3 days later or on a daily basis for 31-days and killed the following day. Dairy cows were killed 3 days after both the single acute dose and the 31 day chronic exposure. Single-combed White Leghorn pullets, male and female Yorkshire swine, and a Holstein dairy cow received a single dose of 17.3 Ci (0.443 mg), 96 Ci (2.46 mg), and 1200 Ci (30.69 mg), respectively, of ^{14}C-naphthalene by oral intubation. The laying pullets (three per time point), approximately 41 weeks of age, were individually caged and fed a complete, all-mash laying ration and water ad. libitum. The growing swine (three per time point), approximately 20 kg, were maintained in individual holding units, and the Holstein dairy cow, approximately 3 years of age, was maintained in an individual paddock area.

In the chronic exposure studies, laying pullets, swine, and a dairy cow received 1.39 Ci/d (0.036 mg/d), 4.36 Ci/d (0.112 mg/d), and 200 Ci/d (5.115 mg/d), respectively, for 31 days. All animals in the chronic experiments were maintained as described above for the acute studies. At sacrifice the following tissues were taken from all animals--two muscle samples, liver, fat (pelvic), heart, spleen, kidneys, and lung. Tissues were weighed and sampled in triplicate, and the samples were placed directly into standard scintillation vials to which 1 ml of tissue solubilizer (Unisol, Isolab, Inc., Akron, Ohio), was added. Upon 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. (In the remaining text, "naphthalene" refers to ^{14}C-naphthalene and/or its various metabolites.)

RESULTS AND DISCUSSION

Results obtained with laying pullets following an acute and chronic exposures to naphthalene show the major tissue site of deposition was the kidney, followed by fat, lung, and liver (Table 1). These data show that naphthalene was readily taken up by the major tissues. The two muscle samples, white (M. pectoralis thoracica) and dark (M. peronaeus longus) meat, indicate that naphthalene is readly taken up by these tissues within 24 hr after exposure whereas approximately 75-80% is eliminated 48 hr postexposure. It is interesting to note that for the two types of muscle, the dark meat apparently retained more naphthalene than the white meat. Although levels of naphthalene were found in lungs at 24 hr, the amount decreased substantially (10%) by 72 hr. The lung values for naphthalene in the chronic exposure studies were much lower, which implies that recontamination via inhalation of the volatile naphthalene was not the cause of the initial high values.

Table 1. Distribution of naphthalene or metabolic products in tissues of laying pullets (% total dose/g tissue 10^{-3})[a]

Tissue	Acute Dose 24 hours	Acute Dose 72 hours	Chronic Dose 31 days
Liver	8.75±0.39	0.96±0.12	0.74±0.10
Fat	13.50+2.00	1.60±0.06	0.37±0.11
Dark Meat	2.63±0.26	0.64±0.21	0.33±0.04
White Meat	1.68±0.16	0.30±0.03	0.16±0.01
Heart	3.91±0.39	0.43+0.03	0.44±0.06
Spleen	3.76[b]	1.36[b]	0.71[b]
Kidneys	42.90±1.05	8.03±1.07	2.40±0.22
Lungs	13.50[b]	2.93[b]	1.24[b]

[a]–mean ± SE
[b]–one sample

The mechanism of fat absorption in avians appears to differ from that in mammals; the hormone enterogastrone is released from the intestine of mammals when fat is absorbed and is presumed absent in birds (Sturkie, 1976). This mechanism may influence naphthalene fat deposition since pullets have a greater 24-hr uptake in fat than swine and dairy cattle and a greater loss at 72 hr. It is hypothesized that some naphthalene was able to pass through the portal system and avoid oxidation in the liver, possibly being bound to lipoproteins in the blood or to membranes, thus accounting for its high deposition in fat.

The uptake of naphthalene by eggs after laying pullets' acute exposure to naphthalene is shown in Figure 1. Within the first 24 hr, detectable amounts of naphthalene were incorporated in both the yolk and albumen (white) of the egg. Internal yolks, referring to yolks at various stages of development, are released one at a time at maturity for subsequent egg formation. These values not only show that yolk is a preferred site of deposition within the egg, but more importantly, signify that eggs will still contain residual amounts of naphthalene after 72 hr. The chronically exposed pullets (Figure 2) show that within the first 24-hr postexposure to naphthalene a significant amount of naphthalene was incorporated into yolk and albumen; the amount decreased with time and reached an equilibrium in the yolk after approximately 20 days. The reason for the decrease in naphthalene levels is not clear, since in the acute study the internal yolk was a primary site of deposition and the levels of naphthalene remained high for 72 hr. Distribution of naphthalene in tissues from chronically exposed pullets shows that liver has approximately twice the activity of fat, which is opposite of results obtained in the acute study (Table 1). Dark meat always retained more naphthalene than white meat. In both the acute and chronic studies, no adverse effects were noted on egg production or body weight.

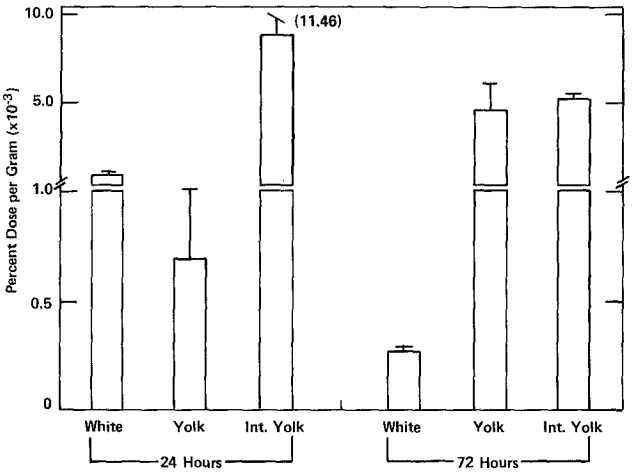

Figure 1. Percent of dose of naphthalene in eggs after an acute exposure. Each bar represents the mean ± standard error of three eggs.

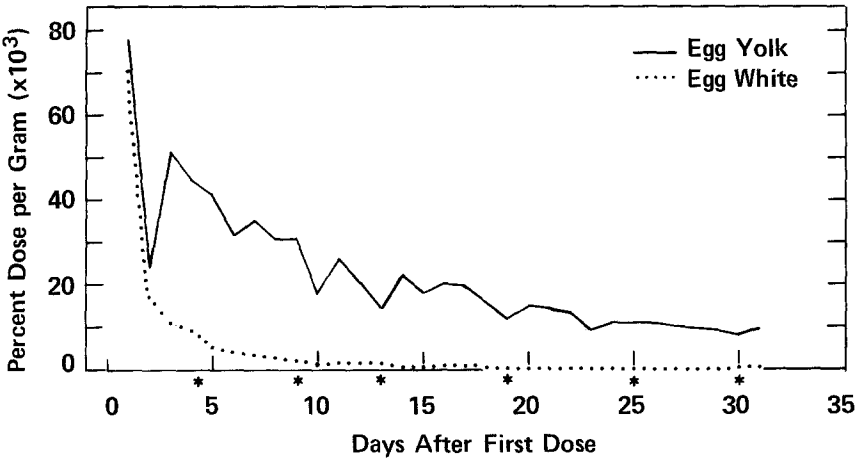

Figure 2. Percent of dose of naphthalene in eggs during a chronic exposure (31 days). Each day represents the mean of three eggs and * indicates mean from two eggs.

After growing swine's acute exposure to naphthalene, the major site of deposition was fat (Table 2), which remained high at 72 hr after exposure. Fat was approximately 10 times higher than liver, which was higher than any of the other tissues. Both the loin (M. longissimus dorsi) and ham (M. biceps femoris) muscles retained low levels of naphthalene (0.12% x 10.3 of dose), and by 72 hr after exposure these levels were reduced by approximately half.

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

Tissue	Acute Dose 24 hours	Acute Dose 72 hours	Chronic Dose 31 days
Liver	0.26±0.06	0.34+0.24	0.11±0.05
Fat	3.48±2.16	2.18±1.16	0.03±0.01
Loin	0.11±0.03	0.05±0.00	0.05±0.00
Ham	0.12±0.02	0.06±0.00	0.06±0.00
Heart	0.09±0.04	0.05±0.00	0.11±0.03
Spleen	0.07±0.01	0.06±0.02	0.09±0.05
Kidneys	0.96[b]	0.26[b]	0.09[b]
Lungs	0.16[b]	0.26[b]	0.15[b]

[a]mean ± SE
[b]one sample

The kidney values 72 hr after exposure were reduced by a factor of 4 with a slight increase in the liver, suggesting possible redistribution of naphthalene between tissues. These results indicate that pork fat used as an additive in pork as well as other nonpork meat products would contribute to contamination of certain food products with naphthalene.

The lung, liver, and heart of swine were the major sites of deposition following a chronic exposure of naphthalene, with little naphthalene being retained by fat. These results are opposite those obtained in the acute study. It is interesting to note that heart muscle had a higher retention value, approximately twice that found in the loin and ham muscles. The reason for these differences is unknown. Also, the acute 72-hr muscle values were similar to the chronic 31-day values.

The results of dairy cows' acute and chronic exposure to naphthalene are shown in Table 3. The liver was one of the major tissues of deposition and differences were observed between the two muscles (loin - M. longissimus dorsi, flank - M. biceps femoris) sampled at 24 hr but not after 34 days. Kidney fat, however, had the lowest retention of naphthalene of the tissues analyzed. Tissue distribution of naphthalene was comparable in both the acute and chronic study in cattle. Lactating cows have little subcutaneous fat; the major fat deposits are located around the kidney. Due to the dynamics of lactation, this fat deposit is a source of energy and the low quantities of fat could possibly be the reason for the low levels of naphthalene observed. The percent of dose administered found in the milk after an acute exposure is shown in Figure 3. Within 8 hr after exposure, naphthalene was detected in milk and equally distributed on a gram basis between milk fat and milk. This distribution remained, although the level for each component decreased throughout the

Table 3. Distribution of naphthalene or its metabolites in tissues of dairy cows (% dose/g tissue 10^{-3})

Tissue	Acute Dose[a] 72 hours	Chronic Dose[a] 34 days
Liver	0.015	0.006
Fat	0.001	0.001
Loin	0.008	0.003
Flank	0.016	0.002
Heart	0.010	0.004
Spleen	0.010	0.004
Kidneys	0.010	0.002
Lungs	0.012	0.003

[a]one sample

72-hr period investigated. Milk values for the cow exposed chronically (Figure 4) again show naphthalene to be essentially equally distributed between milk and milk fat. After the 31 daily exposures to naphthalene, the cow was milked for 3 additional days until sacrifice (34 days). The data show that during this 3-day period the clearance from milk was quite rapid.

In trying to determine the significance of the food chain as a vehicle for toxic agents, it is imperative to characterize the materials entering the system with the appropriate food-producing animals. Although the species evaluated (laying pullets, swine, dairy cattle) were different in tissue distribution and concentration, these data demonstrate that there is an accumulation and retention of naphthalene or its metabolites in consumable meats, eggs, and milk. These products constitute a major portion of many human diets. For example, American consumers eat well in excess of 200 pounds of meat and poultry per capita per year. Dairy milk is utilized as fresh milk and cream, in various processed forms such as cheese, ice cream, butter, and dried milk products with a long shelf life, and in pharmaceutical, industrial and livestock feed products. Milk is a major constituent in the newborn infant's diet and through early childhood. Eggs are also used extensively and in products as an add-in ingredient. Therefore, it is important that the levels of contaminants that might be found in these animal products be accurately determined.

The environmental contamination level of a chemical can only be considered as an indicator of the potential levels in the animal. Much of the body burden of these animals will come from ingested feed and water. The capability of biomagnification, depending on the chemical, may allow animals to biochemically concentrate relatively small quantities of the chemical(s) and accumulate them in the body at levels greater than those encountered in the

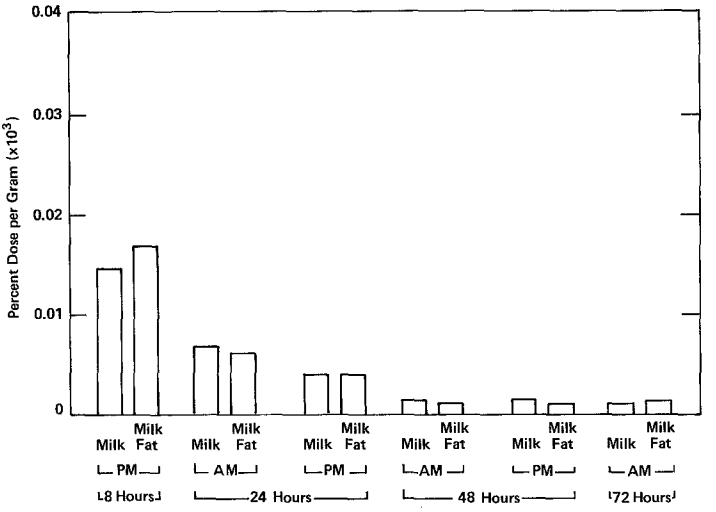

Figure 3. Percent of dose of naphthalene in milk of a dairy cow after an acute exposure. The bars represent the values from one dairy cow.

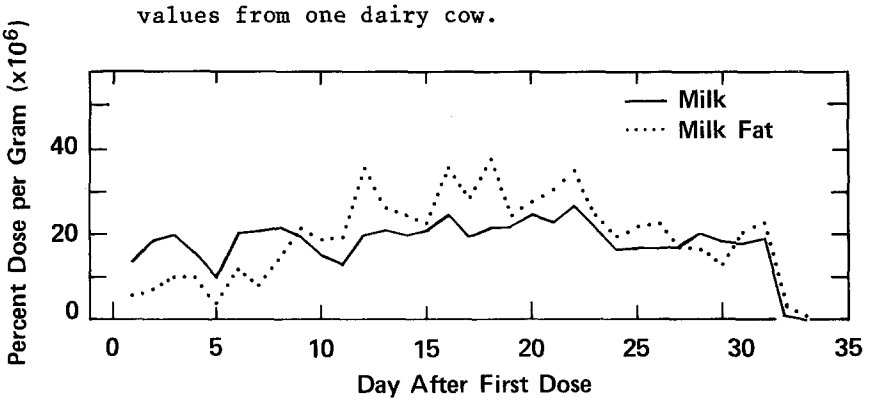

Figure 4. Percent of dose of naphthalene in milk of a dairy cow during a chronic exposure (31 days). Each day represents the mean of the morning and evening milking.

environment. The species studied will also influence retention levels and tissue distribution. For example, fat content of tissue is quite variable not only with regard to tissue type, but also with respect to species age, sex, nutrition, etc. Triacylglycerols in poultry are predominant in fat depots under the skin and in the body cavities, whereas phospholipids and other lipophilic substances comprise approximately 5% of the lipids in certain vital organs such as the liver and heart. This difference in lipid distribution may explain some of the differences in body retention values observed in the three species.

Almost the entire animal is utilized either directly (consumable products) or indirectly (animal feed additives, etc.). Liver, heart, kidney, spleen, etc., are all utilized in meat food products because of their high content of excellent quality protein and essential vitamins. The concentration of naphthalene in the tissues and by-products evaluated in this study, although low, was present and would enter the human food chain.

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