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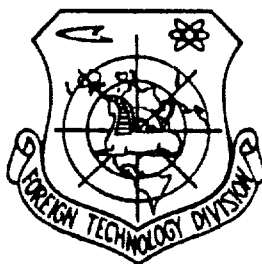
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CORRELATION BETWEEN THE CONCENTRATION OF POLYCYCLIC CARCINOGENS IN
FOODSTUFFS OF ANIMAL ORIGIN AND IN THE FEED OF AGRICULTURAL ANIMALS

by

N.D. Gorelova, P.P. Dikun, et al.



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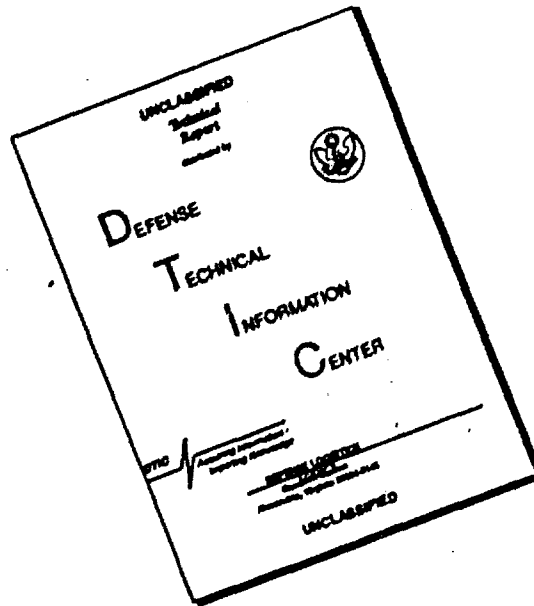
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By: N.D. Gorelova, P.P. Dikun, et al.

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Block	Italic	Transliteration	Block	Italic	Transliteration
А а	<i>А а</i>	A, a	Р р	<i>Р р</i>	R, r
Б б	<i>Б б</i>	B, b	С с	<i>С с</i>	S, s
В в	<i>В в</i>	V, v	Т т	<i>Т т</i>	T, t
Г г	<i>Г г</i>	G, g	У у	<i>У у</i>	U, u
Д д	<i>Д д</i>	D, d	Ф ф	<i>Ф ф</i>	F, f
Е е	<i>Е е</i>	Ye, ye; E, e*	Х х	<i>Х х</i>	Kh, kh
Ж ж	<i>Ж ж</i>	Zh, zh	Ц ц	<i>Ц ц</i>	Ts, ts
З з	<i>З з</i>	Z, z	Ч ч	<i>Ч ч</i>	Ch, ch
И и	<i>И и</i>	I, i	Ш ш	<i>Ш ш</i>	Sh, sh
Я я	<i>Я я</i>	Y, y	Щ щ	<i>Щ щ</i>	Shch, shch
К к	<i>К к</i>	K, k	Ъ ъ	<i>Ъ ъ</i>	"
Л л	<i>Л л</i>	L, l	Ы ы	<i>Ы ы</i>	Y, y
М м	<i>М м</i>	M, m	Ь ь	<i>Ь ь</i>	'
Н н	<i>Н н</i>	N, n	Э э	<i>Э э</i>	E, e
О о	<i>О о</i>	O, o	Ю ю	<i>Ю ю</i>	Yu, yu
П п	<i>П п</i>	P, p	Я я	<i>Я я</i>	Ya, ya

*ye initially, after vowels, and after ъ, ь; e elsewhere.
When written as ѐ in Russian, transliterate as yě or ě.

RUSSIAN AND ENGLISH TRIGONOMETRIC FUNCTIONS

Russian	English	Russian	English	Russian	English
sin	sin	sh	sinh	arc sh	sinh ⁻¹
cos	cos	ch	cosh	arc ch	cosh ⁻¹
tg	tan	th	tanh	arc th	tanh ⁻¹
ctg	cot	cth	coth	arc cth	coth ⁻¹
sec	sec	sch	sech	arc sch	sech ⁻¹
cosec	csc	csch	csch	arc csch	csch ⁻¹

Russian English

rot curl
lg log

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Page 61.

CORRELATION BETWEEN THE CONCENTRATION OF POLYCYCLIC CARCINOGENS IN
FOODSTUFFS OF ANIMAL ORIGIN AND IN THE FEED OF AGRICULTURAL ANIMALS.

N. D. Gorelova, P. P. Dikun, A. P. Dmitrochenko, N. D. Krasnitskaya,
A. I. Cherepanova, I. A. Shendrikova.

Rabbits, pigs, cows, chicken and ducks received during different periods of the year (one year) fodder containing 3,4-benzopyrene. Animals and poultry were kept for 24 hours on termination of the experiment and their tissues and organs analyzed for benzopyrene content. Meat and skin of the animals were examined. Liver and kidney were also taken for analysis. Milk of experimental cows was used for 24 hours, while meat, liver and blood were then subjected to examination. Investigations also covered milk of experimental cows and chicken eggs. Control assays were a common practice as well. Meat and other objects under examination either contained no 3,4-benzopyrene at all, or demonstrated merely its traces. Hence, the presence of relatively large amounts of benzopyrene does not cause this carcinogen to pass on animal food products. Studies were also made as to the amount of intact benzopyrene passed from the organism of rabbits, cows, calves and chicken.

To increase the production of dietary protein of animal origin various methods of protein and vitamin supplementation in stock raising are under development. Great significance is attributed to protein-vitamin concentrates, obtained microbiologically from different types of raw material, including nonfood products [1-4]. Meanwhile in some types of feeds carcinogenic polycyclic hydrocarbons may be found. They may be present not only in products of microbiological synthesis and processed nonfood raw material, but also in some forms of byproducts of the food industry, used in livestock raising even in the main feed ration, [5-11]. In connection with this there exists the danger that carcinogenic hydrocarbons, which are

contained in the feeds of agricultural animals will accumulate in their tissues and organs, and also penetrate the milk of cows and the eggs of birds. In this case the feed may be the reason for the presence of carcinogens in the foodstuffs of animal origin.

In a series of studies we attempted to evaluate the degree of reality of the danger indicated. In search for an answer to the indicated question, first of all we turned to the literature on the metabolism of polycyclic hydrocarbons in the organism of animals and the mechanism of carcinogenesis [12].

Page 62.

The metabolism of carcinogenic polycyclic hydrocarbons was studied in a number of works. Studies done before 1951, showed that most of the carcinogens which enter the organism undergo metabolism and removal. In studies by of C. Heidelberger and colleagues [13], carried out with the use of labeled carcinogenic hydrocarbons, a quantitative characteristic was obtained for these processes. In their experiments with labeled 3,4-benz(o)pyrene, 82.8% of introduced radioactivity was eliminated from mouse organism within 24 hours, and about 1% - in the form of the unaltered substance. Approximately 14%, also mainly in the form of products of metabolism, were retained in the organism after 24 hours. At the same time a number of authors noted the capacity of adipose tissue to more actively retain benzpyrene [14-16].

These studies do not give us an unequivocal answer to the question which interests us, since the conditions for conducting them differ from the conditions of feeding animal feeds which contain carcinogenic substances. We are interested in the possibility of the accumulation of carcinogens in the organism of agricultural animals with systematic prolonged feeding of such feeds. Available literature data were obtained mainly under conditions of a single administration (more often intravenously) of such substances to laboratory animals (rats, mice, sometimes rabbits). In connection with a number of biological characteristics in the process of digestion in different animals [17, 18] and species-specific differences in the metabolism of foreign substances in living organisms [19] it cannot be stated in advance that the process of the metabolism of carcinogenic hydrocarbons will occur equally in laboratory and in various agricultural animals.

Somewhat closer to experimental conditions necessary for solving the above problem were the experimental conditions of Rigdon and Neal [5, 20, 21]. These authors studied the total fluorescence of tissues and organs of rats, mice, hens and ducks during prolonged (up to 30 days) addition to the feed of different doses of benzpyrene. There was noted rapid removal of the ingested carcinogen and the absence of its accumulation in the organism of animals and birds, especially in small daily doses. However, because of the nature of the specific procedure used, the authors could not trace the fate of the unaltered carcinogen. Consequently, even these experiments do not completely

satisfy the requirements, which must be satisfied for solving the stated problem.

We approached experimental resolution of question as follows. First, we studied the tissues of agricultural animals and birds to which feeds which contained carcinogenic hydrocarbons had been fed. Immediate determination of the possibility of accumulation of carcinogens in tissues and organs of animals was the object of this series of studies. Secondly, we studied the products of excretion of the animals and birds. In this case it was intended to compare the metabolism of carcinogenic hydrocarbons received by animals under conditions of prolonged feeding with [metabolism] in the organism of laboratory animals under the conditions of administration used in experimental oncology (acute experiment and introduction of carcinogens mainly intravenously).

Literature data indicate that metabolism of different polycyclic hydrocarbons, carcinogenic and noncarcinogenic, occurs in a single manner [22, 23]. Taking into account this fact, and also the fact that of the strong carcinogens in feeds 3,4- benz(o)pyrene is mainly present, we were interested only in this compound.

On the basis of literature data it is possible to draw conclusion that in process of metabolism of both benzpyrene and other carcinogenic polycyclic hydrocarbons the occurrence of products of metabolism, which possess carcinogenic activity is highly improbable

[24, 25].

Page 63.

In connection with this we considered it worthwhile to restrict our problem to determination of the possibility of accumulation in the organism if unaltered benzpyrene alone.

All the benzpyrene determinations were done employing the procedure used in the laboratory of biophysics of scientific research institute of oncology im. N. N. Petrov. It includes the saponification of tested product, the extraction from it of unsaponifiable part, its chromatographic fractionation, its qualitative and quantitative spectral analysis using quasilinear [quasi-bright-line] fluorescence spectra [26].

In work were used several types of feeds, which contained benzpyrene, sunflower mill cake, nutrient hydrolytic and hydrocarbon yeasts, activated hydrolytic sludge and activated sludge from shale-processing production. Benzpyrene concentration was defined in all these feed specimens, which made it possible to calculate both the daily and cumulative doses received by animals. The results of determining the concentration of benzpyrene in different feeds are given in Table 1.

Experiments for possible concentrations of benzpyrene in tissues and organs were performed on 50 rabbits, 32 pigs, 47 chickens, 10

hens, 36 ducklings, 22 ducks, 3 cows, 2 calves. On the same species of animals several series of experiments were conducted. These differed in duration, daily or cumulative dose of carcinogen, and in certain cases in other ration characteristics of animals. The combined results of experiments are presented in Table 2.

First of all, it is evident from Table 2 that in tests for animals of the same species, no relationship was noted between concentration of benzpyrene in tissues and either one-time nor cumulative dose of the carcinogen. Neither was there found a significant difference in the results from analyses of the tissues of different species of animals.

Table 1. Content of 3,4-benz(o)pyrene in the protein-vitamin feeds [supplements].

(1)	(2)
Feed	
Mill cake, sunflower (3)	
Hydrocarbon yeast (4)	
Hydrolytic nutrient yeast (5)	
Activated hydrolytic sludge (6)	
Shale (7)	

Key: (1). Feed. (2). Content of benzpyrene (in mkg/kg). (3). Mill cake, sunflower. (4). Hydrocarbon yeast. (5). Hydrolytic nutrient yeast. (6). Activated hydrolytic sludge. (7). Shale.

Table 2. Amount of 3,4-benz(o)pyrene, fed in the form of feeds to different animal.

(1) Вид животного	(2) Число исследованных животных	(3) Длительность опыта (в днях)	(4) Максимальная суточная доза бенз(а)пирена на 1 голову (в мкг)	(5) Максимальное суммарное количество бенз(а)пирена, полученное за весь опыт, на 1 голову (в мг)	(6) Среднее содержание бенз(а)пирена, обнаруженное в тканях животных (в мкг/кг)
Кролики (7)	5	2	365	12,1	5,0
Свиньи (8)	2	2	65	3,0	1,9
Куры (9)	12	92	15,0	141,2	0,0—0,04
Птицы (10)	1	1	70	0,07	0,0—0,18
Утки (11)	1	1	55	2,7	0,0—0,02
Гуси (12)	1	1	70	2,8	0,0—0,18
Коровы (13)	8	18	6,0	11,6	0,0—0,22
Лошади (14)	1	18	1,04	7,2	0,0—0,2
Овцы (15)	1	1	5,0	2,0	0,0—0,18
Плоды (16)	1	1	3,0	1,0	0,0—0,18
Плоды (17)	1	1	8,0	1,0	0,0—0,18

Key: (1). Species of animals. (2). Number of investigated animals. (3). Duration of experiment (in days). (4). maximum daily dose of benzpyrene on the average per 1 head (in μg). (5). Maximum total amount of benzpyrene, received during entire test, per 1 head (in mg). (6). Concentration of benzpyrene, discovered in tissues of animals (in mkg/kg). (7). Rabbits. (7a). up to. (8). Pigs. (9). Experiment. (10). Chickens. (11). Hens. (12). Ducklings. (13). Ducks. (14). Cows.

Page 64.

In all 147 analyses of tissues and organs of animals and birds in experiment and 74 in control were performed. Negative results

(benzpyrene not discovered) were obtained in 96 analyses (66%) in the experiment and in 61 analysis (82%) in the control. One should take into consideration that the procedure used by us made it possible to reliably determine benzpyrene in a quantity of 0.004- 0.005 μg or more in the specimen. In conducting the analysis we usually selected 0.2-0.5 kg of tissue. Consequently, only concentrations of from 0.01 to 0.02 mkg/kg can be considered reliable. Among the obtained positive results significant part (31 of 64) was within the limits background values, i.e., it was not reliable. The number of reliable positive results is thus only 15% in the experiment and 11% in the control.

Thus in the overwhelming majority of analyses both in control and in experiment benzpyrene in tissues was either entirely absent or its content was below sensitivity thresholds of the method. Only in a small number of cases was it possible to speak of its real presence in the tissues. In this case, on one hand, such positive results were noted not only in the experiment, but also in the control. On the other hand, the highest concentration of benzpyrene in the tissues was not more than 0.2-0.3 mkg/kg, which is considerably lower than concentration of benzpyrene, for example, in smoked fish and sausage products.

Thus, the feeding even of comparatively large doses of benzpyrene (up to 1.5 mg in a 24 hour period for pigs and 0.8 mg for ducklings) does not lead to the appearance of this carcinogen in meat.

From hens (data on which are cited in Table 2) eggs were

collected at different times and subjected to analysis. As can be seen from Table 3, in the individual analyses both in the experiment and in the control a small quantity of benzpyrene was discovered, which differed little from the background. All these data are within reliable limits. Relationships between the results of analysis and daily or cumulative doses were not observed. Obviously, the presence of benzpyrene in the feed of hens did not lead to its accumulation in the eggs.

We investigated also milk and milk products from two cows (Table 4) and samples of milk, intended for sale (Table 5).

As can be seen from Tables 4 and 5, in milk and dairy products an insignificant quantity of benzpyrene can sometimes be present. However, its content does not rise with the addition to the feed of supplements containing a significant quantity of benzpyrene. It is possible to conclude that the utilization of supplements containing benzpyrene, does not lead to the occurrence in the milk of the carcinogen.

In process of the study metabolism tests on rabbits, hens and cows were performed.

Feces were studied (in experiments on rabbits and cows - urine also) within different periods after beginning of feeding of animal and birds feeds which contained benzpyrene. Activated hydrolytic and

activated shale sludge were used as supplements. The daily doses of benzpyrene for hens was 5.9 and 434 μg , for rabbits 86 μg , for cows about 10 mg. Analyses were done once a month, in experiments on rabbits - over a period of 5 months, on hens - over a period of 4 months. In experiments with cows the analyses were done prior to beginning and 80 days after the beginning of the feeding.

Table 3. Results of determining 3,4-benz(o)pyrene in the eggs of hens.

(1) Добавка к основному рациону кур	(2) Максимальная суточная доза бензпирена в среднем на курицу (в μg)	(3) Суммарная доза бензпирена в среднем на курицу (в μg)	(4) Число исследованных яиц	(5) Количество бензпирена (в μg) на 10 яиц
(6) Шрот подсолнечниковый	—	—	3	0,0—0,17
(7) Шрот из гидрокарбон дрожжей	1,4	2,8	4	0,3—0,09
(8) Гидролизат	4,2	124,0—6,16	135	0,0—0,07
(9) Гидролизат ил	4,5	251,0—632,0	9	0,0—0,05
(10) Сланец	63,0	2 218,0—5 685,0	100	0,0—0,02

Key: (1). Additive to the basic ration of hens. (2). Maximum daily dose of benzpyrene on the average per hen (in μg). (3). Cumulative dose of benzpyrene on the average from hen (in μg). (4). Number of investigated eggs. (5). Quantity of benzpyrene (in μg) per 10 eggs. (6). Sunflower meal. (7). Hydrocarbon yeast. (8). Hydrolytic. (9). Hydrolytic sludge. (10). Shale.

Page 65.

In the feces benzpyrene was found in all cases both in experiment and in control, in urine - only in a few cases. In the control experiments a small amount of benzpyrene was found. In the feces the level of benzpyrene toward the end of the feeding period, as a rule, increased: whereas 30 days after the beginning of experiment it was less than 1% of daily dose, then toward the end of the experiment it rose to several percent. In feces of one of the experimental cows the content of benzpyrene 80 days after the beginning of experiment reached 24% of the daily dose. The content of the studied carcinogen in the urine of rabbits and cows was insignificant and did not depend

on the duration of the experiment.

Results of these experiments showed that from organism of agricultural animals and birds, only a small part of the carcinogen which entered it is eliminated in an unaltered [intact] form, which on the whole will agree with literature data, obtained in experiments on laboratory animals. In keeping with the data presented above, which indicates the absence of accumulation in the organism of unaltered benzpyrene, the results of these experiments show that the carcinogenic substances undergo metabolic transformations.

At the same a relationship was noted between the amount of benzpyrene in feces and duration of feeding supplements, and also its very high concentration in feces of one of cows, indicating that in quantitative sense the metabolism of carcinogen can be affected both by the conditions of the experiment and by the individual, perhaps even species-specific, characteristics of the animals.

Table 4. Results of determining 3,4-benz(o)pyrene in the milk and milk products from experimental cows.

(1) Коровы кормили	(2) Продукт	(3) Содержание бензпирена (в мкг на 1 л продукта)			
		(4) основ- ной рацион	(5) основной рацион + сляк + выр или		
			30 дней (6)	60 дней (6)	90 дней (6)
(7) Фауна	(8) Молоко	0,11	Не обнаружено	0,2 (9)	Не обнаружено (9)
	(10) Сливки		0,01	Не обнаружено	0,03
(12) Бритва	(11) Обрат		0,002	0,15	0,004 (8)
	Молоко	0,04	0,002 (9)	0,002 (9)	Не обнаружено
	Сливки		Не обнаружено	Не обнаружено	0,01 (9)
	Обрат		0,002	0,01	Не обнаружено

Key: (1). Name of cow. (2). Product. (3). Amount of benzpyrene (in μg per 1 l of product). (4). basic ration. (5). basic ration + shale sludge. (6). ... days. (7). Fauna. (8). Milk. (9). Not found. (10). Cream. (11). Skim milk. (12). Britva.

Page 66.

Conclusions.

1. In experiments on rabbits, hens, ducks, pigs and cows using several types of protein-vitamin supplements containing 3,4-benz(o)pyrene in concentrations of from 6 mkg/kg to 43 mg/kg, in overwhelming majority of cases benzpyrene in tissues and organs of animals in was not discovered. Only in a few analyses was it revealed in small quantities which reliably exceeded background level. No correlation was noted between the concentration of benzpyrene in tissues and organs of animals, its concentration in feeds, and the duration of their feeding.

2. Analogous results were obtained also in studying chicken eggs and cow's milk. Benzpyrene either was absent from these products or it was revealed in an insignificant amount both in the experiment and in the control.

Results of experiments indicate that prolonged use of feeds which contained even significant doses of benzpyrene did not lead to its accumulation in organism of animals or in milk and eggs. Metabolic experiments showed that the ingested carcinogenic polycyclic hydrocarbons undergo in the organism metabolic conversions.

Table 5. Results of control analysis of milk.

(1) Проба	(2) Содержание бензпирена (в мг на 1 л молока)
Молоко, бутылочное для продажи	
проба а	0,01
" б	0,02
(4) " в	0,01
Молоко, разлитое для продажи	
проба а	(5) Не обнаружено
" б	0,01
(6) " в	0,01
Контроль реактивов	
проба а	(5) Не обнаружено
" б	(5) Не обнаружено

Key: (1) . sample . (2) . concentration of benzpyrene (mg per l of milk) .

(3) . bottled milk for sale: sample . . . (4) bulk milk for sale (unbottled):

sample . . . (5) . none found . (6) . monitor for reagents .

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