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ORIGINAL ARTICLE

The Toxic Effect of Rhodamine B In Rats

by

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Abstract

A multigeneration ratfeeding study was carried out with rhodamine B incorporated in the usual stockdiet for albino rats.

Rhodamine B is a red inedible synthetic dyestuff wrongly but widely used in Jakarta for colouring cheap snacks and drinks prepared at the homelevel and sold by streetvendors.

Under the conditions of the experiment the main findings observed were :

1) *retardation of growth, starting from birth to adulthood*
2) *decrease in reproductive ability as seen from continuing decrease of littersize with each following generation*
3) *behavioral changes of adult rats, such as itching, irritability, aggressiveness and cannibalism*
4) *increased susceptibility to infection (respiratory diseases) in adult rats resulting in decreased longevity*
5) *development of tumors in the lymphnodes*

The purpose of this investigation is through reporting the findings to awake renewed interest on the part of the authority for the problem of provision of the so badly needed cheap but edible foodgrade colours for the less privileged members of the society.

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Introduction

The use of colouring substances in food is since long a worldwide accepted practice. The food industry using high technology and observing good manufacturing practice, undoubtedly will comply with the existing regulations concerning the use of food colours. Not so is, however the case with the small subsistance producers of cheap snacks and drinks, offered for sale at the sidewalk for the less well-to-do segment of the urban population and especially their children. The indisciminate use of inedible dyestuffs such as for example rhodamine B, metanil yellow, auramin 0 in the preparation of foods and drinks represent a serious health hazard. However it is realized that the solution of this problem is not an easy matter. To arouse renewed interest in this problem a multigeneration ratfeeding study with rhodamine B, a red inedible dyestuff was therefore undertaken with the hope that the obtained findings will add in substantiating the challenge for improvement.

Material and methods

Experimental Diet

Rhodamine B powder, a product from Poland, bought from an importing firm in Jakarta was mixed with the stock diet (addendum 1) at a concentration of 1 gram per 3 kilograms of dry diet (equivalent to 210 milligrams of pure rhodamine B.)

The rhodamine B – mixed diet was fed freely to the experimental albino-rat daily for 6 generations.

Experimental Animal

Albino rate used in this study were from our own stock colony, viz. the Nutrition Research Unit Diponegoro, Ministry of Health, Jakarta, known as "Lembaga Makanan Rakyat (LMR)–strain, Wistar derived".

At the start, 6 female adult albino rats were selected. They are divided equally into 3 cages with each cage containing one adult male rat. After three days to allow for mating, the female rats were separated and housed individually.

During the whole pregnancy and nursing period, the mother-rats (F_0) were fed freely on rhodamine B – mixed diet.

The first offsprings were weighed at monthly intervals and designated as F_1. At the age of 3 months, six female and six male rats were selected from F1 to serve as the successive parent stock for the second generation designated as F_2. This procedure was repeated untul F_6 was obtained.

Each generation of these rats was observed until the age of 12 months for clinical signs and pathological changes.

A control group using the same procedure and same number of rats in each generation was always included.

Results

Clinical signs

In all the experimental rats of the six generations, the hair coat was pinkish coloured due to contact with the dyestuff in the diet. From the age of 6 months onwards besides the pinkish discoloration, the fur became rough and staring. Alopetic areas were also observed occasionally in one or two animals (Figure 1).

In all the rats given rhodamine B, the mucosa of the conjunctivae of the eyes were pinkish red and fluorescent if exposed to sunlight. The eyes were also secreting pinkish tears. Dry pinkish scab was observed in the nasal corner of the eye. From the age of 11 months, most of the animals started to scratch the eyelids, which became swollen and inflamed.

The activity of the young rats (in all generations) given rhodamine B was not much different from that of the respective controls. Some of the adult test-rats became aggressive and cannibalism was observed. However, starting from the age of 8 months a decreased activity of the experimental rats were seen; they became lazy, schedule themselves in a corner of the cage and drunk more water.

The urine and faeces of all the test rats were pinkish in color and fluorescent when exposed to sunlight.

The average number of newborn rats per litter from each generation is shown in Table 1. It can be seen that there is a gradual decrease of the litter size in the rhodamine B rats i.e. from 7.8 in F_1 to 5.4. in F_4 and 3.3. in F_6, while in the control groups the litter size has remained constant through the 6 generations with an average of 7.7.

The birthweights of the newborns in the rhodamine B groups in all of the 6 generations showed a slight reduction as compared to the control groups i.e. 4.7 grams and 5.4 grams respectively.

The average bodyweight gain of the young rats (0–3 months) in the rhodamine B groups was lower than that of the control groups. At 3 months of age, the females and the males in all generations showed an average bodyweight of 111.5 grams and 129.0 grams respectively. In the control groups the average weights were slightly higher viz. 121.9 grams in the females and 135.0 grams in the males.

During pregnancy, the average total weight increase of the adult female rats of the rhodamine B group was lower than that of the controls. It is observed that after the age of 9 months, the weights of the female rats progressively decrease until the end of the experiment, by the time they were 12 months old. In the adult male rats from the age of 9 months the body weights also decreased until the end of the experiment (12 months old) like in the case of the females.

The final average body weights of each of the 6 generations recorded at the end of each experiment were 149.0 grams in the females and 188.0 grams in the males; while in the female and male controls 211.0 grams and 258.4 grams respectively. The body weight changes expressed in per cent of starting weight can be seen graphically in Figure 2 and Figure 3.

Mortality rates

The average mortality rates for the group of 0 – 3 months old rats for all generations as compared to the controls is presented in Table 2. The average mortality rate observed in the age group 0 – 3 months for the 6 generations is 5.3% for the control and 31.5% for the rhodamine B fed group. The average age specific mortality rate (3 months to 12 months) were zero% for the control and 63.9% for the rhodamine B fed rats. No difference was found between the mortality rate of male and female rats fed rhodamine B.

Death was due to increased susceptibility to respiratory disease (pneumonia); 63% of the adult experimental rats died at the age of 12 months while no death was observed in the control group. Neoplastic growth (tumours) were found in 6 experimental rats (3 females and 3 males), located in the mediastinal as well as in the mesenterial lymph nodes (Figure 4). Microscopically this tumour tissue was diagnosed as a lymphoma.

In the control groups, tumour development was not observed (Table 3).

Discussion

As has been observed from the results of this experiment, the toxic effects of rhodamine B included: roughened haircoats, swollen eyelids, fluorescent urine, decrease of litter size, slight reduction of birthweight, lower bodyweight gain and abnormal bodyweight changes, early death, high mortality rates, and the development of tumours in some rats as compared to controls.

A question may arise what effects will be seen especially in young children who are consuming the pink coloured snacks or drinks daily. Probably no one will know the answer but it seems wise and prudent to remind the consumers about the public health hazard of this dyestuff.

The mere fact that rhodamine B is an inedible colouring matter (not permitted in foods and drinks) is already an indication of its hazardousness or toxicity for human health.

The abnormal findings observed in ra' may serve as a stimulant to prevent the use of non-permitted inedible food colours.

Even the consumption of edible permitted food colours may have toxic effects, if the quantity per unit of food or the permissible level is not observed, as illustrated by the following historical case. A candy company had made Halloween candy using the edible FD and C Orange No. 1 to match the colour of pumpkins. A number of children who ate this candy had severe gastrointestinal upsets. This incident could happen as at that time (under the 1938 Act of the Food and Drug Admiinistration (FDA - USA) the upper limit of the use of colouring matters in foods was not yet established (Loren, 1969). With the aforementioned information in mind, one may wonder what will be the effects of rhodamine B in foods and drinks, the more as it is an substance and yet used in unknown or uncontrolled concentrations in various snacks and drinks in Jakarta. As mentioned before according to Sihombing (1979), the rhodamine B content in 35 coloured snacks had a very wide range from 2.56 mg to 23.75 mg per 100 grams of product. Moreover one may wonder about the presence of other contaminants such as heavy metals in the synthetic dyestuffs (Jacob, 1973), especially in these inedible colouring agents never intended for human food use.

Why are some of the inedible colouring matters such as rhodamine B used instead of the permitted food colours available in the market? The home-food-producers prepare the snacks and drinks on consumer's demand and try to obtain the cheapest price but with the highest profit from their products. Conceivably, they will use the cheapest ingredients which will give attractive products. Rhodamine B can give such an attractive effect when mixed with various kinds of ingredients such as flour, coconut milk, and sugar (common ingredients used for making home-made Indonesian snacks). The end product, whether it is fried, baked or steamed will be more appealing and bright. As thery are home-food-producers, they are not registered at the Ministry of Health and/or Department of Commerce, and therefore beyond the reach of any authority control. Consumers in general do not know either the way to choose safe foods. As long as the foods are tasting good, and they do not give a bitter or strange taste, furthermore they looked attractive and within their buying power, they will buy it.

They shopkeepers in the marketplace who are selling colouring matters either for dyeing clothes or for colouring foods also want to satisfy their customer but with profit in their mind and are less interested in how it is used. These shopkeepers buy attractive colouring agents of cheap price from the wholesale distributors, repacked the substance into bottles or plastic bags and after adding fillers put labels on the container such as "Special Colour for all Purposes". Some of the labels are com-

pleted with a picture of fruits (Sihombing, 1978). This picture gives the false impression that the substance is for food use which is in fact totally misleading.

The price of these colouring agents is much cheaper i.e. about one fourth of that of the permitted foodgrade colouring matters, also available from wholesale distributors. This low price must therefore be another reason why the inedible rhodamine B is used in various snacks and drinks in Jakarta markets.

The observation on the toxic effect as seen in the rats and the information presented above may serve as a clear warning to halt the dangerous use of rhodamine B in human foods. This can be achieved through a cooperated action involving several governmental ministries and private institutions such as Yayasan Lembaga Konsumen; Public education through schools and mass media (radio, TV, newspapers etc.) in order to give correct information about the proper use of colouring matters especially those considered a necessity for colouring foods and drinks. Besides, import duties levied for food colours should be as low as possible.

Conclusion

The abnormal findings observed in our experimental multigeneration rat feeding study may serve as a new stimulant in the effect to reduce the inappropriate use of nonpermitted inedible colours in foods.

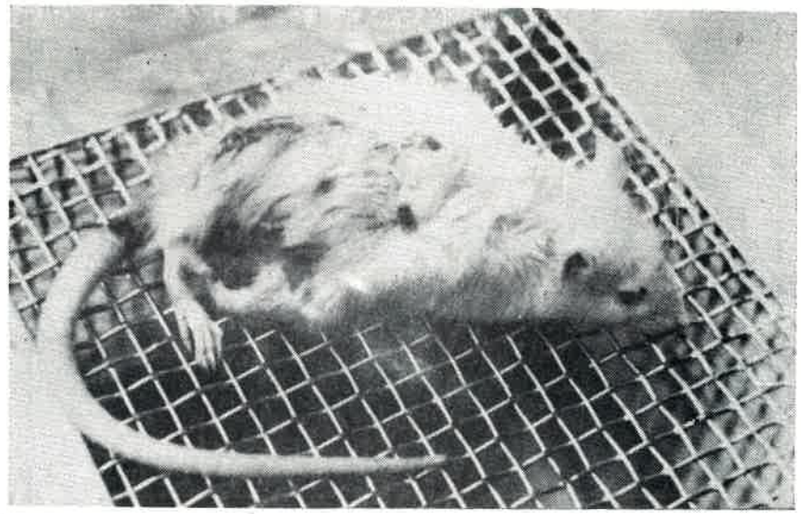
The mere fact that rhodamine B, metanil yellow, auramin 0 etc. are inedible colouring matters and obviously not permitted in foods, indicates already its hazardousness or toxicity for human health. Yet its continued use by the home foodproducers belonging to the lower socio-economic class, points out the demand and necessity of colouring matters for foods. The homeproducers haverestored to the use of the synthetic non-permitted inedible colours most certainly because of its cheap price and its easy availability in the market supported by a distribution system which is not geared to bear responsibility for the health of the public.

Education alone of all those involved in the distribution system and all those involved in the consumption system, will not be sufficient as long as there is a demand for synthetic food colouring matters. The provision of foodcolours should be made to satisfy the demand of the public, who as described above are of low economic stratum. In other words, the edible foodcolours to be made available must be within the buying capacity of the users/consumers. Education of not only of the groups concerned but he public in general also in regard to the dangers of nonpermitted inedible colours when used in food coupled also with the proper use of permitted foodcolours should be intensified.

Acknowledgement

I wish to express my thanks to Dr. Iwan T. Budiarso for his cooperation throughout this study; to Dra Oey Kam Nio and Dr. Lie Goan Hong for their expert guidance and encouragement.

URE 1. *Rhodamine B fed rat. Note alopetic areas seen in the hind quater of the body and tail*



GURE 4. *Rhodamine B fed rat. Note tumour nodules at the mediastinal and mesenterial lymph nodes (arrows).*

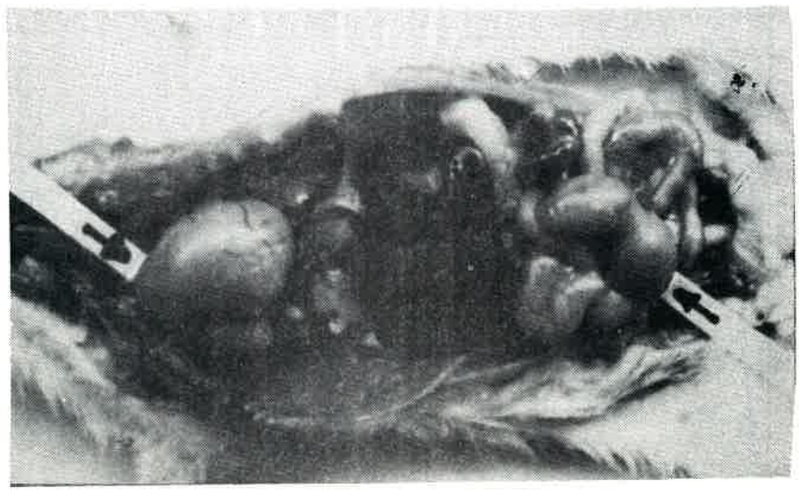


FIGURE 2. *Body weight changes of female-rats expressed in per cent of starting weight (3 months old) fed rhodamine B mixed with food in a 12–month–study.*

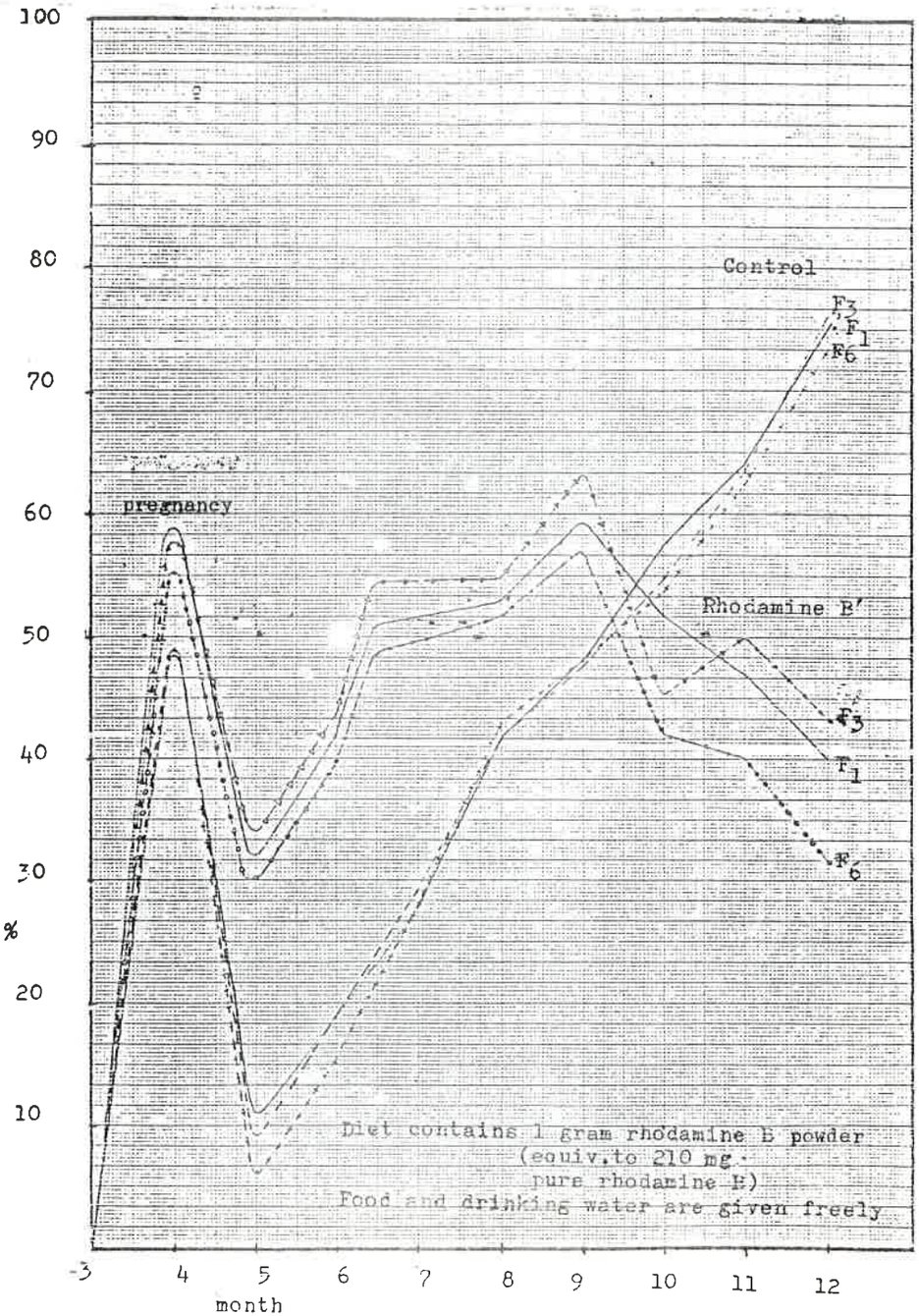


FIGURE 3. *Body weight changes of male-rats expressed in per cent of starting weight (3 months old) fed rhodamine B mixed with food in a 12–month–study.*

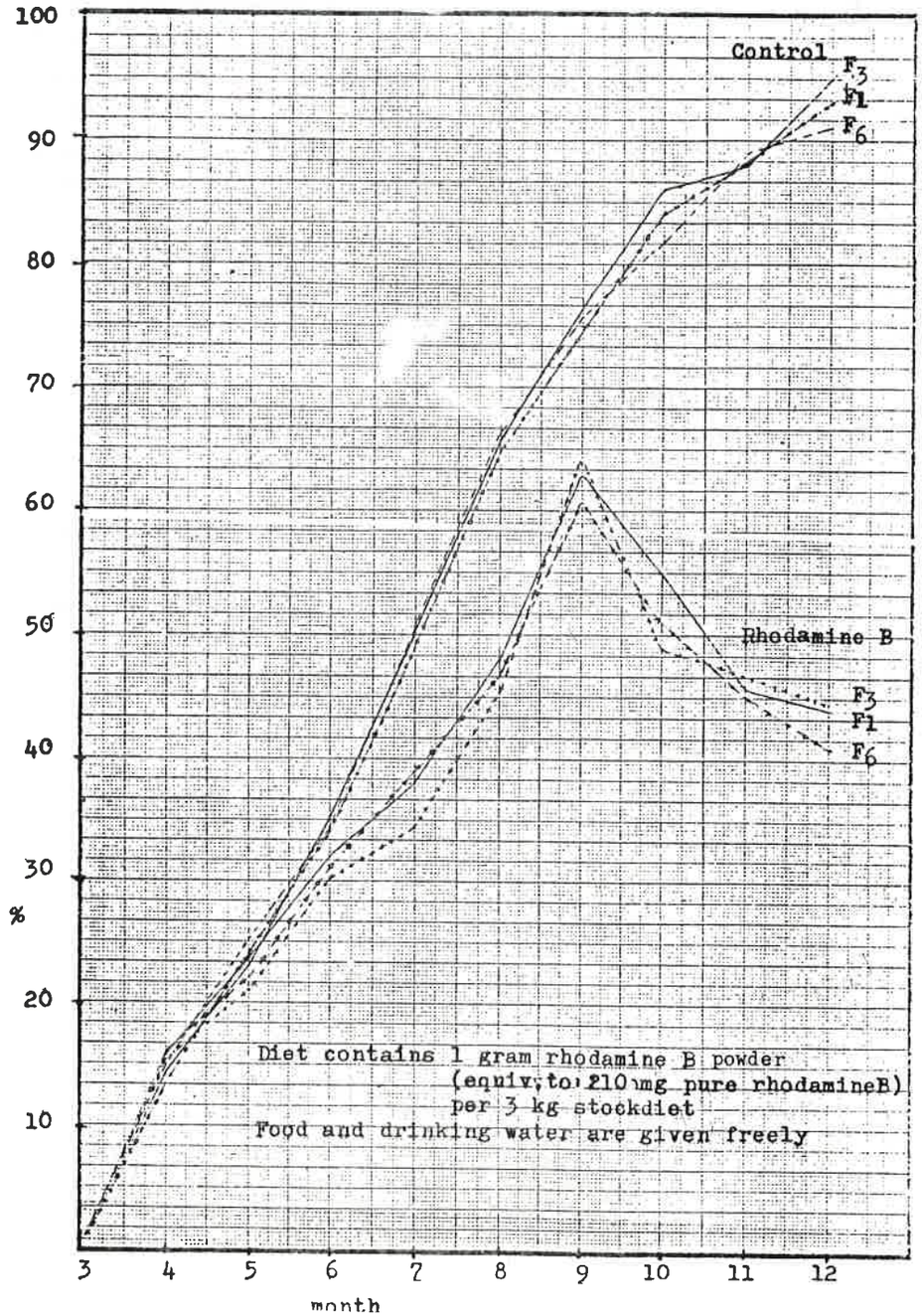


TABLE 1 : *Average littersize of rats fed on rhodamine B observed over 6 generations* *

Generation F	Number of mother rats in each generation	Average number of newborn rats	
		Control	Fed on rhodamine B
F_1	6	8.1	7.8
F_2	6	7.5	6.6
F_3	6	7.8	5.7
F_4	6	7.7	5.4
F_5	6	8.0	3.3
F6	6	7.0	3.3
Average		7.7	5.4

* Newborn rats were recorded at time of birth.

TABLE 3 : *Number of survivals and dead adult rats ; tumour development in rats fed on rhodamine B mixed in food observed during 6 generations* **
(observation of 1 generation lasted for 12 months)

Generation		Number of Surviving rats. Month												Number of Deaths at End in %	
	Female	at start 3 months of age	IV	V	VI	VII	VIII	IX	X	XI	XII	Total	%
F_1	Female	6	6	5	4	4	2	1	0	0	0	0	6	100
	Male	6	6	6	6	6	4(1*)	2	1	1	1	16.7	5	83.3
F_2	Female	6	5	4	3	3	2	2	2	2	2	33.3	4	66.7
	Male	6	6	6	6	6	6	5(1*)	4	3	2	33.3	4	66.7
F_3	Female	6	6	4	4	4	2(1*)	2	2	2	2	33.3	4	66.7
	Male	6	6	6	6	6	6	5	4	3	2	33.3	4	66.7
F_4	Female	6	6	4	2(1*)	2	2	2	2	2	2	33.3	4	66.7
	Male	6	6	6	6	5	4	3	3	3	3	50.0	3	50.0
F_5	Female	6	6	6	6	6	4(1*)	4	3	3	3	50.0	3	50.0
	Male	6	6	6	6	6	6	5	4	3	3	50.0	3	50.0
F6	Female	6	6	6	6	6	6	6	6	5	4	66.7	2	33.3
	Male	6	6	6	6	5	5	5	4	4	2(1*)	33.3	4	66.7
Average	Female	36												63.9
of Total	Male	36												63.9

* Found tumour developing, diagnosed as lymphoma

** All controls were alive until the end of experiment, death was 0.

TABLE 2 *Comparison of age (0 – 3 months) mortality rates of albinorats fed rhodamine B and Control-diet over generation*

Generation	Number of rats	Control: Total young rats at birth	Control: Number of death during 0 – 3 months		Fed – Rhodamine B: Total young rats at birth	Fed – Rhodamine B: Number of death during 0 – 3 months	
	n	n	number n	%		number n	%
F_1	6	49	3	6.1	47	14	29.8
F_2	6	45	3	6.7	37	17	45.9
F_3	6	47	2	4.3	31	15	48.4
F_4	6	46	1	2.2	27	6	29.6
F_5	6	48	4	8.3	20	4	20.0
F_6	6	46	2	4.4	20	3	15.0
Average of 6 generations				5.3 %			31.5 %

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Addendum 1.

Composition of Prepared Stockdiets for Albino-Rats, Strain I.M.R., wistar derived.

	Basic Foodstuff : Protein %	Fat %	Weight in kg	Weight in %
1. Rice	7.0	1	10.0	54.3
2. Soybean, boiled, dried	40	18	4.5	24.4
3. Peanut, shelled, fried	27	44	1.5	8.1
4. Skim milk powder, high quality	35	–	2.0	10.8
5. Coconut-oil	–	100	250 ml	1.3
6. Kitchensalt	–	–	0.15	0.8
7. Bonemeal	–	–	0.075	0.4
8. Vit. B–complex tablet *			30 tab	+
9. Vit. A + D_3 in starch **			+	+
10. Ferri-citrate			+	+
			± 18.4 kg	± 100%

Average Composition as calculated :

Crude Protein	19.6 %
Total Fat	9 %
Total Energy	370 Cals.%
NPU-standard	60
NPU-operative	50

as analysed : Crude Protein 20.3 %

For Comparison : Composition of Purina Laboratory Chow :
(Ralston Purina Co. Louis, USA.)

Crude Protein	23.0 %
Nitrogen free extract	50.6 %
Crude fat	3.8 %
Crude fibre	4.9 %
Ash	7.7 %

* B–complex,
Each tablet contains :

Thiamin HCl	3	mg
Riboflamin	2	mg
Pyridoxin HCl	0.5	mg
Calcium Pantothenate	2	mg
Nicotinamide	10	mg

** Rovimix A + D3 Type 500/100 Roche :
1 gram contains 500.000 IU Vit. A + 100.000 IU Vit.D

– 125 grams Rovimix to 400 grams of starch
– For 18.4 kg of food use 8 grams of (Starch + Rovimix). (equiv. to 0.25 g Rovimix).

Source : Nutrition Research Unit Diponegoro
National Institute for Health Research and Development,
Ministry of Health, Jakarta.