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Provisional translation

Evaluation Report

MADDER COLOR

July 2, 2004
Food Safety Commission
Food Additives Expert Committee

(Progress of Evaluation)

June 18, 2004	The Ministry of Health, Labor and Welfare (MHLW) requests health risk assessment in line with withdrawal of Madder color from the list of existent food additives
June 24, 2004	50th meeting of Food Safety Commission (explanation of MHLW's request outline)
July 2, 2004	10th meeting of Food Additives Expert Committee 52nd meeting of Food Safety Commission (reporting of discussion results from the Food Additives Expert Committee and being finalized) The Food Safety Commission reports the result of assessment to MHLW
July 5 to 30, 2004	Public comments
August 19, 2004	58th meeting of Food Safety Commission (reporting of results of public comments)

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Evaluation Report of Food Additive “Madder Color”

1. Introduction

“Madder color” is a coloring agent extracted from madder root (*Rubia tinctorum* L.), which has been allowed as one of the existing food additives in Japan[*1, *2].

In other countries, it is allowed to use as a food additive in Korea but not in the United States and European Union (EU).

*1 Existing food additives were already marketed or used on the date of the amendment of the Food Sanitation Law in 1995 and appear in the List of Existing Food Additives.

*2 As one of coloring agents its use is limited for kind of kelp, meat, fresh fishes and shellfish (including meat of whales), tea, kind of laver, beans, vegetables and kind of wakame.

This evaluation was conducted since the Ministry of Health, Labor and Welfare (MHLW) requested evaluation of the health risk concerning withdrawal of Madder color from the List of Existing Food Additives to the Food Safety Commission on basis of the Food Safety Basic Law. (Its related documents were received on 18th June, 2004.)

2. Outline of this substance

Name: Madder color (extracted from madder (*Rubia tinctorum* L.) root, and its major components are alizalin and ruberythric acid)*

Properties: Stable to heat or light, soluble in water and ethanol
Taking on yellow under acidic condition, and on red under neutral one

Use: Coloring agent

* Including lucidin-3-*O*-primeveroside, ruberythric acid, alizalin, etc.

3. Summary of the Toxicological Studies

(1) Genotoxic Studies

In the DNA repair test using *Bacilus subtilis*, Madder color (including Madder root, Glycoside mixture, Rubia Teep® and Madder powder extracted with ethyl acetate) showed negative by protocol widely used but weakly positive by the liquid method at extremely high concentration in the absence of an exogenous metabolic activation system.[1)] In the bacterial reverse mutation assay using *Salmonella typhimurium and Escherichia coli*, the positive responses were reported in the presence and absence of an exogenous metabolic activation system. [1)-5)] No data on chromosomal aberration in mammalian cells was

available to the expert committee.

As an *in vivo* assay, wing spot test in *Drosophila melanogaster* was negative.[6] In DNA adduct formation tests *in vivo*, it was reported that Madder color formed DNA adducts in the liver, kidney, duodenum and colon.[7), 8)] For chromosomal aberration induction, the mouse bone marrow micronucleus test was carried out up to substantial high doses, Madder color gave a negative result.[9]

(Lucidin-3-*O*-primeveroside and Lucidin)

Positive responses were reported for lucidin-3-*O*-primeveroside that is one of major components of Madder color in both reverse mutation test in bacteria[10] and UDS test in primary rat hepatocytes[11]. Also regarding lucidin, positive results were reported with reverse mutation test in bacteria[3), 4), 10), 12), 13)], DNA single-strand breaks test[12], UDS test in primary rat hepatocytes[11], gene mutation test in mammalian cells[12] and transformation test in C3H/M2-mouse fibriblasts[12]. It was reported that lucidin showed DNA adducts in the liver, kidney, duodenum and colon of mice.[7] While, the chemical did not induce gene mutation in *Drosophila* wing spot tests.[6]

(Alizarin, etc.)

Some results were reported regarding other components of Madder color such as alizarin, but the results were conflicting.[3), 6), 7), 10), 11), 13)]

Considering these data, regarding lucidin, many positive data have been reported in gene mutation tests, which is considered enough evidence. However, genotoxic data of madder color *per se* was limited. And regarding chromosomal aberration test of madder color, there is only negative data in the mouse micronucleus test but no more data *in vivo*, which is considered limited information to conclude.

There are many positive data on lucidin, but most of them are *in vitro* test system. On the other hand, only positive result *in vivo* was reported for DNA adduct formation test, the other assays, i.e., the somatic mutation and recombination in *Drosophila melanogaster* resulted negative.

Although it is considerable that DNA adduct formation was reported in the kidney, which was a target organ of carcinogenicity, there is no evidence that the initial event had been fixed to gene mutation and/or chromosomal aberration. There is possibility that its genotoxicity would play a part of role for carcinogenicity, but at the present time it is difficult to conclude that there is enough evidence to ensure it.

(2) Acute Toxicity

LD_{50} was determined >5000 mg/kg bw for acute toxicity (14 days) in SD rats.[14] LD_{50} was determined >5000 mg/kg bw for acute toxicity in $B6C3F_1$ mice.[15]

(3) Subchronic Toxicity

In 90-day toxicity study (0.6, 1.2, 2.5 and 5% in diet) with F344 rats, histopathological changes were observed in the kidney in males at doses of 1.2% and higher and in all the treated females, and in the liver in females at the dose of 5%. No observed adverse effect level (NOAEL) was considered to be 0.6% in males and less than 0.6% in females. [16)]

In 90-day toxicity study (0.3, 0.6, 1.25, 2.5 and 5% in diet) with B6C3F$_1$ mice, no adverse effect was observed in both males and females. [15)]

(4) Chronic Toxicity/Carcinogenicity

A medium-term multi-organ carcinogenesis bioassay was investigated, in which F344 rats treated with diethylnitrosamine, *N*-methylnitrosourea and *N*-bis(2-hydroxypropyl) nitrosoamine were administered two types of madder color at dietary levels of 2.5% and 5% for 16 weeks. No tumor promoting effect was observed in any organs. [17)]

In 780-day repeated dose (1 and 10%) carcinogenicity test with ACI/SegHsd rats, tumors were observed in the liver and kidney but the incidences were not statistically significant. [8)]

The results of Combined chronic toxicity/carcinogenicity test (progress report) [18)] presented this time are summarized as follows:

Chronic toxicity and carcinogenicity of madder color (major components: lucidine-*O*-primeveroside, ruberythric acid and alizalin) were investigated in F344/DuCrj rats.

In the chronic toxicity test, 10 males and 10 females were administered madder color (0, 0.2, 1.0 and 5%) for 53weeks. At the dose of 5%, atypical tubules were seen in both males and females, and decreases food consumption, increased serum creatinine, formation of renal cell adenoma (1 case) and dilated medullary sinus of mesenteric lymph nodes were observed in males. In both sexes at doses of 1% and higher, some hematological and serum biochemical paramaters changed, but no histopathological changes related to the treatment were detected in the spleen, bone marrow, liver, bone and heart. An increase of absolute kidney weights, vacuolar degeneration of cortical proximal tubular epithelia, anisokaryosis of proximal tubular cells of the outer medulla were observed. Tubular regeneration and infiltration of mononuclear cells to the interstitum were evident in males at doses of 1% and higher. NOAEL of this test was considered to be 0.2% in both males and females.

In the carcinogenicity test, 50 males and 50 females were administered madder color (0, 2.5 and 5%) for 104weeks. Incidence of renal cell carcinomas significantly increased in males at the dose of 5%. A significant decrease of body weights and significant increases of kidney weights and incidences of anisokaryosis of proximal tubular cells, atypical tubules and renal cell adenoma were observed in both males and females at doses of 2.5% and higher. It is reported that histopathological examination is still in progress.

4. Evaluations in the International Organizations

Joint FAO/ WHO Expert Committee on Food Additives (JECFA) has not evaluated Madder color.

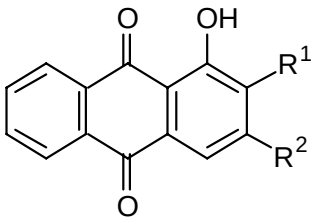
In the report of International Agency for Research on Cancer (IARC) in 2002[19], carcinogenicity of 1-hydroxyanthraquinone (identified as a metabolite of lucidin primeveroside, found in *Rubia tinctorum*, when lucidin primeveroside was given orally to rats) and madder root (*Rubia tinctorum*) was evaluated. 1-Hydroxyanthraquinone was evaluated as Group 2B (possibly carcinogenic to humans), while madder root (*Rubia tinctorum*) as Group 3 (not classifiable as to its carcinogenicity to humans).

5. Production and Import

It is reported that production of Madder root was ca. 5 ton in the 2002 fiscal year and ca. 3 ton in the 2003 fiscal year. Presuming population of Japan to be 127 million, the simple averages of these data correspond to 0.1 mg/person/day and 0.06 mg/person/day, respectively.

The import of Madder color is not reported in Japan, but the amount of import of foods Madder color is added to is reported to be ca. 40 ton in the 2002 fiscal year and ca. 23 ton in the 2003 fiscal year.

Cf. Structure of Major Components of Madder Color



	R^1	R^2
Lucidin-3-*O*-Primeveroside	CH_2OH	O-Glu[1]-[6]Xyl
Ruberythric acid	O-Glu[1]-[6]Xyl	H
Alizalin	OH	H
Lucidin	CH_2OH	OH
Rubiadin	Me	OH

6. Evaluation

Though it is necessary to collect toxicological data on organs/tissues other than the kidney, Madder color has been shown to be genotoxic as well as carcinogenic to the rat kidney from the available data. The Food Additives Expert Committee of the Food Safety Commission concluded that no ADI. (Acceptable Dairy Intake) could be established for this substance.

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