EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Directorate C - Scientific Opinions
C2 - Management of scientific committees; scientific co-operation and networks

UPDATE OF THE OPINION OF THE SCIENTIFIC COMMITTEE ON ANIMAL NUTRITION ON THE SAFETY OF PRODUCT NIFURSOL®

(Adopted on 17 March 2003)

1. BACKGROUND

The product "Nifursol®" has already been assessed by SCAN. The Committee concluded that the safety for the consumers could not be ensured on the basis of the data made available for the evaluation.

The Company submitted a supplementary dossier in reply to the SCAN opinion.

2. TERMS OF REFERENCE

The Scientific Committee for Animal Nutrition (SCAN) is requested to consider the supplementary dossier provided by the company on Nifursol® and to answer the following questions:

(1) Do the supplementary data from the depletion studies and from the mutagenicity studies on the genotoxic potential of Nifursol® allow the establishment of the ADI and the human exposure to Nifursol® residues (including metabolites)?

(2) Can the safety of Nifursol® to the human consumer be ensured?

3. SUMMARY OF THE PREVIOUS SCAN OPINION ON NIFURSOL

The Scientific Committee on Animal Nutrition issued an opinion on product Nifursol® in October 2001[1]. On the basis of the data made available to the Committee, the following conclusions were drawn:

---

[1] Opinion of the Scientific Committee on Animal Nutrition on the safety of use of Nifursol in feedingstuffs for turkeys of 11 October 2001, available at http://europa.eu.int/comm/food/fs/sc/scan/index_en.html

3.1. Mutagenicity

Taken as a whole, the results of *in vitro* testing indicated that Nifursol® had a mutagenic potential in the presence of metabolic activation. Nifursol® possibly also had mutagenic potential in the absence of any metabolic activation, although this may be due to metabolism by bacterial nitroreductase. The results of *in vivo* mutagenicity studies that used bone marrow as the target tissue (cytogenetics and micronucleus assays) were clearly negative. None of the *in vivo* studies that used other target tissues gave convincingly negative results, even if the negative result from a limited carcinogenicity bioassay gave some reassurance. Only the provision of reassuring results from further *in vivo* mutagenicity studies using two different target tissues could allay concerns arising from structural alerts and positive results in some *in vitro* assays.

3.2. Carcinogenicity

The available data did not give a clear indication of any tumourigenicity from Nifursol®. However, there were short-comings in the design of the study and in the absence of details of histopathology, including tumour data from individual animals, the conclusions were regarded as provisional.

This conclusion was reviewed by SCAN on the basis of some histopathological data of 1970 on carcinogenicity made available by the Company which confirm the conclusions on carcinogenicity, which from provisional were then considered definitive[2].

3.3. Residues

No identification of tissue residues was performed. No investigation on the absorption, distribution and excretion of Nifursol® was conducted. No study was performed using repeated dosage with radiolabelled Nifursol® that would allow to establish the tissue residue steady state then depletion, target tissue and marker residue.

The Committee examined an additional residue study of 1967 submitted by the company. This was a global kinetic study carried out using C^{14}-labelled Nifursol® and based on the measurement of the whole radioactivity in the tissues. Although these results answered part of the requests of the SCAN, they did not satisfy all the requirements.

According to the Guidelines a kinetic study of the depletion of unchanged Nifursol® and major metabolites (above 10%) in the edible tissues following Nifursol® withdrawal is mandatory in order to identify the target-tissue and marker-residue. The quantitation of metabolites and eventual identification for

---

[2] Summary Record of the 141rst SCAN Plenary Meeting (Brussels, 05-06 February 2002) (Approved on 17-18 April 2002), available at http://europa.eu.int/comm/food/fs/sc/scan/index_en.html

those representing more than 10% of the total residual radioactivity is a pre-requisite to that study.[3]

3.4. Acceptable Daily Intake

The conclusions drawn from the mutagenicity, genotoxicity studies, as well as the non-conclusive results of a chronic toxicity study in dogs and the lack of data on developmental toxicity, and, moreover, the fact that only one metabolic route is common to the turkey and rat, do not allow to fix an ADI for the human consumer.

4. DOCUMENTS SUPPLIED IN RESPONSE

Despite the exchanges with the company consecutive to the SCAN opinion of October 2001, the concerns expressed by the Committee remained, in particular its genotoxic potential and the absence of a kinetic study of residues.

On 12 February 2003, the Company, Solvay, has responded to the SCAN Opinion on Nifursol® by sending in some additional data, including:

- Nifursol®: Evaluation of the possible induction of *lacZ*⁻ mutations in tissues of treated Muta™Mice
- A Nifursol® depletion study in turkeys
- Preparation of Nifursol® metabolites 1,2,3 and 4
- Determination of Nifursol® and two metabolites in turkey skin, muscle, kidney and liver using LC-MS: method validation
- Determination of Nifursol® and metabolites in turkey skin, muscle, kidney and liver using LC-TIS/MS/MS: depletion study sample analysis

5. SCAN OPINION

5.1. Mutagenicity study

A study was performed in transgenic mice, Muta-Mice, with the aim to determine the ability of Nifursol® to cause mutations to the *lacZ* transgene in ileum/jejunum and liver tissues. After initial dose-range-finding studies to determine the maximum tolerated dose (MTD), groups of eight male Muta-Mice were given daily oral gavage doses of 0 (control), 550 or 850 mg Nifursol® /kg bw for 28 days. The highest dose was the MTD. The mice were killed for necropsy at three days after the last dose. DNA was extracted from the ileum/jejunum and the liver of each animal. Unfortunately the liver samples were contaminated with Nifursol® and therefore could not be investigated. DNA from the ileum/jejunum was introduced into *E. coli C lac⁻ galE⁻ Kan^r (galE⁻ Amp^r)* bacteria using a bacteriophage lambda vector. The bacteria were plated out in the presence of phenylgalactose to detect any *lacZ*⁻ mutants. Counts of the bacterial colonies showed no increases in the numbers of mutants at either dose level, as compared with the control group. It was

[3] Summary Record of the 142nd SCAN Plenary Meeting (Brussels, 17-18 April 2002) (Approved on 18-19 June 2002) available at http://europa.eu.int/comm/food/fs/sc/scan/index_en.html

concluded that Nifursol® did not induce *lacZ* mutations in the ileum/jejunum of the Muta–Mouse.

The SCAN Opinion had requested a further *in vivo* mutagenicity study in two different tissues other than bone marrow, using a test other than the UDS assay. The results for the intestines were clearly negative, those concerning the liver were uninterpretable. The results of this new study therefore do not adequately respond to the SCAN request.

Nifursol® gave negative results for mutagenicity in a range of studies in different tissues: bone marrow and intestines. The genotoxicity seen in some *in vitro* studies was not expressed *in vivo*. Although the weight of evidence from all the mutagenicity studies on Nifursol® suggests that this substance is not an *in vivo* mutagen the missing information requested by SCAN should be provided to reach a final conclusion.

5.2. Residues studies

In its opinion of October 2001 the SCAN concluded that the human exposure to Nifursol® residues (including metabolites) could not be established, due to the fact that the identification of tissue residues was not performed and that the target tissue and marker residue were not established.

The petitioner has supplied additional data intended to address the outstanding issues.

A depletion study of Nifursol® in turkeys (Kan, 2003) consisted in the determination of Nifursol® and two metabolites in the tissues of male and female turkeys fed a Nifursol® supplemented diet (75 mg/kg). The GLP phase started at the age of about 8 weeks and lasted for 7 weeks. Six males and 6 females were then slaughtered after 0, 3, 5, 7, 9 and 12 days withdrawal.

The first metabolite, 5-nitro-2-furoic acid, corresponded to a metabolite already identified in the turkey and rat urine in the metabolic study of Lozano and Morrison (non dated). The second, 3,5-di-nitro-salicylic acid hydrazine-N-glucuronide if one refers to the molecular formula and structure but erroneously noted 3,5-di-nitro-salicylic pyruvic acid-N-glucuronide, corresponded to a metabolite already identified in the turkey urine (not in the rat) in the metabolic study already mentioned. No rationale has been given concerning the choice of these metabolites. An apparent constraint was the availability of standards: 5-nitro-2-furoic acid is commercially available while 3,5-di-nitro-salicylic acid hydrazine-N-glucuronide was the only compound successfully synthesised through attempts made to prepare standards of four potential metabolites of Nifursol® in the chicken (Heres, 2003).

The methodology used to determine Nifursol® and 5-nitro-2-furoic acid was derived from a method already described (De Vries, 1994). That concerning the determination of 3,5-di-nitro-salicylic hydrazine, either free or freed by acidic treatment from 3,5-di-nitro-salicylic hydrazine-N-glucuronide but also from eventual Nifursol® bound residues, was based on a chemical derivatization described to measure nitrofuran bound residues in tissues (Leitner *et al*., 2001).

The global results (no individual values were supplied) indicate that Nifursol® and 5-nitro-2-furoic acid are below the limit of determination (0.02 mg/kg) in the liver, kidney, muscle and fat, whatever the withdrawal time, which confirms data already available (George et al., 1973). When 3,5-di-nitro-salicylic hydrazine is concerned, the results reported indicate very significant levels in all tissues that decrease along the withdrawal period. However, in the absence of the identification of tissue residues in turkey that would prove the existence of Nifursol® bound residues, the search for 3,5-di-nitro-salicylic acid hydrazine as a marker of these bound residues remains purely hypothetical. The fact that this compound may exist also under a free form and as a glucuronide conjugate (metabolite IV already mentioned) would not allow in any case to establish its origin. Moreover, four other potential tissue metabolites that are specific to the turkey have not been investigated. Therefore, the SCAN reiterates that the identification of tissue residues and the characterisation of the target tissue and marker residue (preferably using a radiolabelled compound) remain a pre-requisite for a pertinent evaluation of the human exposure.

In conclusion, the new data brought by the petitioner concerning the depletion of Nifursol® residues in turkey tissues only confirm that unchanged Nifursol® represents a very minor part of tissue residues. The fact that the tissue metabolites and marker residue have not been identified does not allow the SCAN to endorse the scientific approach followed by the petitioner and the other results presented.

6. CONCLUSION

(1) Despite an increasing weight of evidence, the complementary *in vivo* genotoxicity study supplied by the petitioner does not bring all the information required previously and therefore does not allow the SCAN to conclude that Nifursol® is non genotoxic *in vivo*. This, together with the other missing data (see 3.4), does not allow establishing an Acceptable Daily Intake.

(2) The complementary study concerning the depletion of Nifursol® in turkeys does not allow the SCAN to establish the human consumer exposure, and therefore to conclude on the safety of this product. The SCAN reiterates that both the identification of tissue residues and the characterisation of the target tissue and the marker residue are a pre-requisite for the evaluation of the human exposure. Furthermore, in view of the specific metabolic pathway established in turkey, additional relevant toxicological data may be required by SCAN for eventual turkey-specific major metabolites (above 10%) identified in tissues.

7. REFERENCES

De Vries E.J., Bas R. and Kuil H. 1994. Liquid chromatographic determination of Nifursol® in concentrates, Premixes and finished turkey feeds. J.A.O.A.C., 77, 1347-1352.

Heres H.H. 2003. Preparation of Nifursol® metabolites 1, 2, 3 and 4. Syncom, Groningen (NL).

Kan C.A. 2003. A Nifursol® depletion study in turkeys. ID TNO Animal Nutrition, Lelystad (NL).

George GM, Frahm LJ and McDonnell JP, 1973, "Depletion of nifursol residues in turkey tissue from birds medicated with 75 ppm nifursol", Pharmaceutical Development and Analysis Department, Salisbury Laboratories, Charles City, Iowa. Report No. TR-374-73.

Leitner A., Zöllner P. and Lindner W. 2001. Determination of the metabolites of nitrofuran antibiotics in animal tissue by high-performance liquid chromatography-tandem mass spectrometry. J. Chromatogr. A, 939, 49-58.

Lozano JA and Morrison JL, undated, "The metabolism of nifursol (3,5-dinitrosalicylic acid 5-nitrofurfurylidene hydrazine) in the turkey and rat", Company report, Salsbury Laboratories, Research Division, Charles City, Iowa.