EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

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

OPINION OF THE SCIENTIFIC COMMITTEE ON ANIMAL NUTRITION ON THE SAFETY OF USE OF NIFURSOL IN FEEDINGSTUFFS FOR TURKEYS

(adopted on 11 October 2001)

1. BACKGROUND

Nifursol has been authorised at Community level in annex I of Council Directive 70/524/EEC[1], under the conditions set out in Commission Directive 89/23/EEC[2] of 21 December 1988 (see table hereafter).

The SCAN has delivered an opinion in April 1982 (fourth series) on the use of Nifursol in turkeys.

In the veterinary field, all nitrofuranes have been inserted into annex IV of Council Regulation 2377/90/EC (Council Regulation N° 2901/93 and Commission Regulation N° 1442/95). The molecules which have been examined were : nitrofurazone, nitrofurantoin, furaltadone and furazolidone.

Additive	Chemical formula, description	Species or category of animal	Minimum content	Maximum content	Other provisions
			mg/kg of complete feedingstuff		
Nifursol	3,5-dinitro-N′-(5-nitrofurfurylidene) salicylohydrazide Minimum purity: 98% on an anhydrous basis Particular features of the three authorised preparations: - maximum nifursol content: 14.6%, 44% or 50% - minimum stability: 24 months - carrier for the three preparations: maize starch and 12%, 33% or 34% of soya bean oil respectively	Turkeys	50	75	Use prohibited at least 5 days before slaughter Maximum amount of dust emitted during handling, as determined by the Stauber Heubach method[3]: 0.1 µg nifursol

1 OJ n° L 270 of 14.12.1970, p. 1.

2 OJ n° L 11 of 14.01.1989, p. 34

3 Reference: Fresenius Z. Anal Chem (1984) 318: 522-524, Springer-Verlag 1984

2. TERMS OF REFERENCE (MAY 1999)

The Scientific Committee for Animal Nutrition (SCAN) is requested to answer the following questions:

(1) Is Nifursol mutagenic? genotoxic?

(2) Is Nifursol tumorigenic? carcinogenic?

(3) By using Nifursol in feedingstuffs for turkeys, according to the conditions set up by Council Directive 70/524/EEC, is there a risk for the health of the consumer resulting from consuming foodstuffs of animal origin containing residues of Nifursol or of its metabolites?

3. PREAMBLE

Nifursol belongs to the family of nitrofurans, substances harbouring a 5-nitro group, structure which is suspected of imparting genotoxic characteristics to these substances.

The 40th meeting of JECFA (1992) found furazolidone and nitrofurazone, two compounds of this family, to be genotoxic in various *in vitro* tests, but also to cause tumours in rats and mice, with furazolidone causing malignant tumours and nitrofurazone causing benign tumours. JECFA did not identify an Acceptable Daily Intake (ADI) for either of these drugs.

The CVMP considered the safety of various nitrofuran drugs over the period 1990 to 1995. It agreed with the JECFA's toxicological assessment of furazolidone and nitrofurazone. The toxicological data available on other nitrofuran drugs, nitrofurantoin and furaltadone, were too sparse to allow to assess their safety. Therefore the CVMP was not in a position to identify an ADI for any of these nitrofuran drugs. Nifursol was not assessed by the CVMP.

4. MUTAGENICITY AND GENOTOXICITY

4.1. *In vitro* testing

4.1.1. Bacterial Reverse Mutation assay

Nifursol was tested in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 & TA1538 in the presence and absence of S9 from the livers of Aroclor 1254 treated rats. The following chemicals were used as positive controls:

- 10 µg/plate of 4-nitro-o-phenylenediamine (TA 98 & TA1538 without S9),
- 2 µg/plate of aflatoxin B_1 (TA100 with S9),
- 2 µg/plate of N-methylnitrosoguanine (TA100 & TA1537 without S9),
- 10 µg/plate of 9-aminoacridine (TA1537),
- 2 µg/plate of nitrofurantoin (TA100 without S9).

Strains T1535, TA1537 & TA1538 were not challenged with positive control substances in the presence of S9.

In a first experiment, concentrations of 0.2, 2, 20 & 500 µg/plate of nifursol were tested. The variation in the reversion rates was unusually high. The number of revertants in TA98 at 0.2 µg/plate with S9 (mean of 3 determinations) was 23 times more numerous than in the spontaneous reversion rate (mean of 2 plates). This result fitted the criterion for a positive result (more than a 2-fold increase above the spontaneous reversion rate), but higher doses gave negative results. There was a suggestion of a dose related increase with TA1538 without S9: 0, 0, 13 & 21.3 colonies per plate at 0.2, 2, 20 & 500 µg/plate, respectively, whilst the positive (nitrofurantoin) control result was 27 colonies/plate and the spontaneous reversion rate was 15.5 colonies/plate. This may have represented a mutagenic response, but as the positive control did not cause more than a two-fold increase in revertants above the spontaneous rate, the results for this strain remain unclear.

In a second experiment, using the same doses, there was even more variation in results with the numbers of revertants for strain TA1538 with 0.2 and 20 µg/plate in the absence of S9 being greater than those for the respective positive control (4-nitro-o-phenylenediamine). This time however all of the positive controls acted as expected causing clear mutagenic responses in the respective strains. With TA1538 in the absence of S9, 20 µg/plate of nifursol caused a 22-fold increase in the number of revertants in TA1538, as compared with the spontaneous reversion rate, and 0.2 µg/plate caused a 10-fold increase. There was no dose-response relationship.

The author suggested that the two experiments should be considered together rather than individually and concluded that the treatment caused no mutagenicity in any strain. However, the author's conclusion was not supported by the data. There was evidence of mutagenicity in TA98 at the lowest dose in the presence of S9 in one of the experiments and some evidence of mutagenicity in TA1538 in the absence of S9 in both experiments. This result should be regarded as equivocal. [Green, 1980]

A second *Salmonella*/microsome mutation study was reported. Nifursol was tested in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 & TA1538 in the presence and absence of S9 from the livers of male Sprague Dawley rats treated with Aroclor 1254. Positive controls used were 5 µg/plate of N-methyl-N-nitro-N-nitrosoguanidine (without S9), 75 µg/plate of 9-aminoacridine (without S9),

50 µg/plate of 4-nitrofluorene (without S9) and 5 µg/plate of 2-aminoanthracene (with S9). Concentrations of nifursol of 0 (solvent control), 6.7, 33.3, 166.7, 500 & 1500 µg/plate, dissolved in dimethylsulphoxide (DMSO), were used. At the highest dose there was some precipitation of the test material. A statistically significant

(Dunnett's t-test, $p<0.01$) positive response was observed at concentrations of 166.7 and 500 µg/plate in TA100 in the absence of S9, with cytotoxicity occurring at 1500 µg/plate. In the presence of S9, TA100 gave a positive response at 166.7, 500 & 1500 µg/plate. In TA98, there was a positive response ($p<0.01$) at 500 & 1500 µg/plate only in the presence of S9. The positive controls gave clearly positive results as expected. This result indicates that nifursol was mutagenic in this assay.[Cavagnaro & McCarrol, 1985]

Nifursol showed a positive mutagenic response in the presence and absence of S9 and only in strains of *Salmonella* known to have high activities of nitroreductase. It is possible that nitroreduction of nifursol is needed before it has mutagenic activity in bacteria. However testing in nitroreductase deficient strains has not been performed to confirm this.

4.1.2. In vitro cytogenetics assay:

CHO cells were exposed to five concentrations of 2.5, 8.5, 25, 85 & 250 µg/ml of nifursol dissolved in DMSO. The cells were tested in the presence and absence of S9 from an unspecified source. Mitotic division was arrested using colcemid, allowing cells to be examined in metaphase. Two negative controls were used: one consisted of the incubation medium alone (Ham's F-12) and the other was made up of incubation medium plus solvent (DMSO). Mitomycin C (80 µg/ml) was used as the positive control in the absence of S9 and cyclophosphamide (140 µg/ml) was used as the positive control in the presence of S9. The results are summarised in Table 1. The positive controls gave clearly positive results, as expected. There was a statistically significant (Dunnett's one-tailed t-test to a significance of $p<0.05$) increase in the frequency of chromosomal aberrations at the highest readable dose (85 µg/ml) in the absence of metabolic activation (8% of cells contained aberrations, as compared with 1% and 2% in the solvent control and medium controls respectively), but not at other concentrations without activation nor at any concentration in the presence of metabolic activation. The aberrations seen at 85 µg/ml were 6 chomatid breaks, 1 chromosome break, 3 exchanges and additionally there were 6 chomatid gaps (although gaps were not counted as aberrations). At concentrations of 85 µg/ml or greater a precipitate formed when the test material was added to the medium.

Table 1: Results of *in vitro* cytogenetics study: percent of cells containing aberrations

Group	Without S9	With S9
Medium control	2	3
Solvent control	1	3
Positive control	32	36
2.5 µg nifursol /ml	2	2
8.5 µg nifursol /ml	0	4
25 µg nifursol /ml	2	1
85 µg nifursol /ml	8	1
250 µg nifursol /ml	-	2

There was a clear increase in aberrations, including exchanges, at the top readable dose, in the absence of S9. The result of this test is regarded as positive.[Cavagnaro & Cortina, 1985a]

In a second test, using concentrations of 50, 75, 100, 150 & 200 µg/ml, there was a slight but not statistically significant increase (Dunnett's one-tailed t-test: $p<0.05$) in the frequency of chromosomal aberrations at most concentrations, in both the presence and absence of S9. The types of chromosomal aberration found was not reported in detail, although it was stated that most of the aberrations were chromatid breaks. There was no clear dose-response relationship. An increased number of chromatid gaps (not included in the count of aberrations) was seen at 50 µg/ml (12 gaps) and at 75 µg/ml (10 gaps) as compared with the solvent control (3 gaps), but it was not stated whether this referred to the tests with S9 or without S9. The number of gaps in other groups and the number of cells containing chomatid gaps were not reported. It was noted that a precipitate was visible at concentrations of 100 µg/ml or more, in the absence of S9. The positive controls gave clearly positive results.

Table 2: Results of repeat *in vitro* cytogenetics study: percent of cells containing aberrations

Group	Without S9	With S9
Medium control	2	1
Solvent control	2	4
Positive control	91	40
50 µg nifursol /ml	9	4
75 µg nifursol /ml	9	6
100 µg nifursol /ml	5	6
150 µg nifursol /ml	7	8
200 µg nifursol /ml	6	5

This study is difficult to interpret, since full details of the types of aberrations seen were not given and there was an apparent increase in total aberrations at most doses, as compared to negative control groups. However there was no dose-response relationship to the increase in aberrations. The result for this *in vitro* chromosome aberration assay is regarded as equivocal. [Cavagnaro & Cortina, 1985 b]

The overall conclusion about the *in vitro* cytogenetics studies is that there is cause for concern about possible clastogenicity.

4.1.3. In vitro UDS Assay in Rat Hepatocytes:

Isolated rat hepatocytes were exposed to concentrations of 1, 5, 10, 50, 100 & 500 µg/ml of nifursol in DMSO. Then the incorporation of radiolabelled thymidine into the nucleus was assessed by autoradiography. 0.05 µg/ml of 2-acetylaminofluorene was used as a positive control. Precipitation of nifursol was seen at concentrations of 50 µg/ml or more. It was reported that nifursol did not cause unscheduled DNA synthesis (UDS) in the hepatocytes at any of the concentrations used. [Cavagnaro & Sernau, 1985]

4.1.4. Conclusion

Taken as a whole, the results of *in vitro* testing clearly indicate that nifursol has 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.

4.2. *In vivo* studies

4.2.1. In vivo mouse micronucleus assay:

Groups of 5 male and 5 female mice were given single oral gavage doses of 10,000 mg nifursol/kg bw (in an aqueous solution of 1% gum tragacanth), which was the maximum tolerated dose. The polychromatic erythrocytes from the bone marrow were subsequently examined for micronuclei. Mitomycin C was given to a positive control group of mice.

Table 3: Summary of results of the mouse micronucleus assay

Sampling time (hrs)	Treatment	Ratio of polychromatic to normochromatic erythrocytes	Mean incidence of micronucleated polychromatic erythrocytes (per thousand cells)
24	Vehicle control	0.849	0.6
24	Nifursol	0.884	0.8
24	Mitomycin C	0.340	50.1
48	Vehicle control	0.819	1.1
48	Nifursol	1.533	1.6
72	Vehicle control	1.156	0.6
72	Nifursol	2.061	0.7

It was reported that in nifursol-treated rats there was an increase in the ratio of polychromatic to normochromatic erythrocytes (P/N ratio), indicating that the test material had reached the bone marrow. Nifursol did not cause an increased incidence of polychromatic erythrocytes that contained micronuclei. The positive control group had a highly significant increase in the number of polychromatic erythrocytes that contained micronuclei, but the P/N ratio was decreased. It was concluded that this mutagenicity test gave a negative result.[Allen & Proudlock, 1987]

4.2.2. In vivo rat cytogenetics assay:

An *in vivo* cytogenetics assay was performed in rats using a single oral gavage dose of 10,000 mg nifursol/kg bw (in an aqueous solution of 1% gum tragacanth), which was the maximum tolerated dose. Negative control groups were given the gum tragacanth vehicle alone. Groups of 5 male and 5 female rats were killed at 6, 24 & 48 hrs after treatment. Cyclophosphamide (40 mg/kg) was given to a positive control group of rats, which was killed at 24 hrs. Bone marrow cells were examined in metaphase, mitosis having been arrested using an intraperitoneal injection of colchecine.

Table 4: Summary of results of the *in vivo* rat cytogenetics assay

Sampling time (hrs)	**Treatment**	**Incidence of aberrant cells (%) excluding gaps**	**Incidence of aberrant cells (%) including gaps**
6	Vehicle	0	0
6	Nifursol	0.2	0.2
24	Vehicle	0	0
24	Nifursol	0.4	0.6
24	Cyclophosphamide	19.4	20.4
48	Vehicle	0	0
48	Nifursol	0.2	0.2

No aberrant cells were detected in any of the control groups, but a few aberrant cells were detected in bone marrow from nifursol treated rats. However, in Wilcoxon's sum of ranks test, the differences between the nifursol-treated and their respective negative control groups were not statistically significant ($p>0.05$). In contrast, the positive control group had highly significant increases, as expected. The result was interpreted as negative. [Allen, et al., 1987]

4.2.3. In vivo UDS Assay in Rat Liver:

Groups of 3 male Wistar rats were given oral gavage doses of 100, 300 or 1000 mg/kg bw of nifursol (in an aqueous solution of 1% gum tragacanth) and were killed at 2 or 12 hours after dosing in separate

studies. The highest dose was said to be the maximum soluble dose, although the OECD Guideline 486 requires testing at up to a maximum dose of at least 2000 mg/kg bw. This could have been achieved if a micronised suspension had been used or two doses had been given at different times of the day. A vehicle control group was given 1% gum tragacanth. Positive control groups were given oral doses of aqueous solutions of 300 mg/kg bw of methylmethane sulphonate (2 hr exposure study) or 100 mg/kg bw of 2-acetylaminofluorene (12 hr study). 300 mg/kg bw of 4-nitroquinoline-N-oxide (12 hr study). At the end of the appropriate exposure period the rats were killed and primary hepatocyte cultures were prepared from their livers.The incorporation of radiolabelled thymidine into the nuclear material of hepatocytes from treated animals was measured *in vitro* by autoradiography as a test of unscheduled DNA synthesis (UDS). The net nuclear grains detected in both positive control groups was clearly greater than in vehicle controls and the difference was statistically significant in Student's t-test ($p<0.05$). No significant difference in net nuclear grains was seen between vehicle controls and nifursol-treated rats. It was concluded that oral exposure to nifursol did not cause UDS in the livers of nifursol-treated rats. This result should be viewed with caution as the concentrations of nifursol tested were less than those recommended in the internationally accepted OECD Guideline for this assay. [Benford, 1987]

4.2.4. In vivo UDS Assay in Rat Intestines:

Groups of 3 male Wistar rats were given oral doses of 100, 300 or 1000 mg/kg bw of nifursol (in an aqueous solution of 1% gum tragacanth) for 2 or 12 hours exposure periods in separate studies. A vehicle control group was given 1% gum tragacanth. Positive control animals were given an oral dose of 300 mg/kg bw of 4-nitroquinoline-N-oxide in corn oil. The incorporation of radiolabelled thymidine into the nuclear material of intestinal cells (enterocytes) from treated animals was measured *in vitro*.

Table 5: Intestinal cell *in vivo* UDS study: mean values

Treatment	**2 hrs exposure**		**12 hrs exposure**	
	net nuclear grains	**% of cells in repair**	**net nuclear grains**	**% of cells in repair**
Vehicle control	0.52	1.3	0.13	5.3
Positive control	0.67	4.7	11.82	89.6
100 mg/kg bw	0.63	4.3	1.03	11.0
300 mg/kg bw	0.72	5.1	3.04	32.9
1000 mg/kg bw	0.57	4.2	3.91	38.8

It was reported that increased incorporation of tritiated thymidine and percent of cells in repair were seen in enterocytes from animals given

300 or 1000 mg/kg bw with 12 hrs exposure period. The effect was statistically significant (Student's t-test, $p<0.05$) only in the 300 mg/kg bw group. In the 2hrs exposure study, there was no apparent effect on net nuclear grains or percent of cells in repair. A large amount of incorporation of tritiated thymidine was seen in the positive controls with 12 hrs exposure (Student's t-test, $p<0.05$), as expected, but no response was seen with the 2 hrs exposure time. It was suggested that the positive result in this test could be due to either UDS (indicating mutagenicity) or scheduled DNA synthesis due to increased cell turnover in response to irritancy. [Benford, 1987a]

The intestinal UDS assay was followed up by a study designed to investigate whether or not nifursol causes irritation of the gut. Groups of 3 male Wistar rats were given 2 oral doses of 1000 mg/kg bw of nifursol (in 1% gum tragacanth) on successive days. A vehicle control group was given a 1% of gum tragacanth. Positive control groups were given either 200 mg/kg bw of the known irritant, cholic acid, or 300 mg/kg bw of the carcinogen, 4-nitroquinoline-N-oxide. The number of mitoses, gross cellular damage and mucous cell activity were monitored in different parts of the gastrointestinal tract. In was reported that in the intestines in general, and especially in the ileum, nifursol had irritancy potential. Cholic acid gave similar results. Both nifursol & cholic acid caused redness in the stomach after 48 hrs but no other indication of irritancy. It was concluded that high doses of nifursol were irritant to the intestines. It was suggested that the apparently positive result in the intestinal UDS assay may have been due to scheduled DNA synthesis as a result of irritancy, however, it is also possible that nifursol was both irritant and mutagenic. No conclusion can be drawn about the relevance of the results of the intestine cell UDS study to the evaluation of the mutagenic risk from nifursol. [Benford, 1987b]

4.2.5. In vivo DNA Binding Study in Rats

Radiolabelled nifursol (^{14}C-labelled on the methylene group) was administered orally to male Wistar rats at a dose of 200 mg/kg bw in a 1 % (w/v) aqueous solution of gum tragacanth. Vehicle controls received aqueous gum tragacanth alone. Tritiated aflatoxin B_1 (in aqueous gum tragacanth) was used as a positive control substance at a dose of 2 mg/kg bw. Covalent binding to DNA was measured in liver, kidney & intestines at 6 & 24 hrs after dosing.

Table 6: DNA binding: mean results

Tissue	**pmol nifursol derived material per mg of DNA**	
	6 hrs	**24 hrs**
Liver	8.93	11.55
Kidney	4.95	24.35
Intestine	8.20	36.80

In all three organs investigated, small amounts of ^{14}C-radioactivity were associated with DNA at 6 & 24 hrs. The total amount of nifursol-derived material decreased between 6 & 24 hrs in the liver and the intestines but increased in the kidneys. Over the same period, levels of nifursol-derived material bound to macromolecules after solvent extraction decreased in intestines, stayed the same in liver and increased in kidneys. Substantial DNA binding was found in the livers of the positive controls, with lesser amounts in intestines and kidneys. The results of this mutagenicity test can not be interpreted as being clearly positive. The low level of DNA-binding found in all three organs investigated in this study may be a non-specific effect unrelated to genotoxicity. The site of the ^{14}C-label makes it ambiguous whether the results were due to metaoblic incorporation or binding. The most unfavourable interpretation that could be put on these results would be that nifursol may be a weakly genotoxic agent. [Connelly, 1988]

4.2.6. Conclusion

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 gives 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. Normally, an *in vivo* liver UDS assay using a maximum dose of at least 2000 mg/kg bw would be considered as a useful additional study, but in view of the negative result of the *in vitro* hepatocyte UDS study the value of such a study is dubious. One of the newer multi-tissue assays such as the *in vivo* comet assay, looking at several tissues including the stomach, intestines and liver might give more relevant results.

5. CARCINOGENICITY

A long-term toxicity/carcinogenicity study was performed, but the report of the study [Rude, 1970c] did not include histopathology results and tumour incidence data. However, it has been possible to obtain some information about tumour incidences from summary tables reproduced in various other documents [Dawes, 1988; Jens, 1987; Narveson, 1988].

Groups of 40 males and 40 females Simonsen Long-Evans rats were fed nifursol at dietary concentrations of 0, 400, 600, 800 and 1000 mg/kg. The study was continued until mortality reached 30%: 117 weeks for males and 118 weeks for females. Blood samples were taken at 2, 6, 12, 18, 24 and 27 weeks for haematology. The parameters measured in all blood samples were pcv, Hb, rbc, wbc total and wbc differential. No clinical chemistry parameters were measured. Urine samples were

collected in weeks 2, 6, 12, 18, 24 and 27 and were investigated for colour, sediment and specific gravity and were tested for blood, glucose, ketones and protein using a test strip. Animals were killed at the end of the study. Post-mortems were not performed on all of the animals: data were available for only 20 to 32 animals from each group of 40 rats. The criteria used for choosing which rats to examine were not explained. A limited selection of organs (liver, kidneys, heart, spleen & testes) were weighed. The following tissues were taken at post-mortem for histopathology: any abnormal tissue, adrenals, bile duct, brain, caecum, colon, duodenum, heart, ileum, jejunum, kidneys, liver, lung, ovaries, pancreas, pituitary, prostate, spleen, stomach, testes, thyroid, urinary bladder and uterus.

Mortality in the various groups ranged from 7.5% (male controls) to 30% (control females and 800 mg/kg males), but there was no dose-response relationship. It was reported that there were no statistically significant ($p>0.05$ in an unspecified test) differences between controls and treated groups in bodyweight gain and food intake. There were no treatment-related trends seen in haematology or urinalysis results, although there were some age-related changes (protein and leucocytes in urine samples collected at 24 and 27 months).

Organ weights were not increased, but there were decreases in liver weights in 600, 800, and 1000 mg/kg males and 800 mg/kg females. The decreases in liver weight were dose-related in males, but it was claimed that the effect was not associated with any gross pathology or histopathology. Details of the histopathology results were not available. Statistical analysis (by an unspecified test) showed no significant differences between controls and treated groups ($p>0.05$) with regard to gross pathology and histopathology. However, the data on non-neoplastic lesions were not reported in detail.

Tumour data from this study have been examined by several different groups: the initial authors (details of this interpretation were not available), and then statistically reviewed by two laboratories in the USA [Narveson, 1988] and the Netherlands [Dawes, 1988]. Both statistical analyses indicated that the observed incidences of total malignant tumours and of all individual types of malignant tumours were not affected by the nifursol treatment.

For benign tumours, the incidences of several types of tumours, including interstitial cell adenomas, renal tubule adenoma, mammary fibroadenomas, were higher in some treated groups than in the appropriate controls. Both statistical analyses showed a significant positive dose-related trend for renal adenoma in male rats. However the difference between the control and 1000 mg/kg groups of males was not significant ($p>0.1$) in exact tests. The initial report claimed that the apparent excess of mammary fibroadenomas was not statistically significant when corrected for time of death ($p=0.29$ in an unspecified statistical test). Both analyses of the mammary fibroadenoma data showed there to be no statistically significant dose-related effect on the incidence of this type of tumour. The increases in incidence of other tumour types and of total benign tumours were not statistically significant.

Conclusion.

The available data do not give a clear indication of any tumourigenicity from nifursol. However, there are short-comings in the design of the study and in the

absence of details of histopathology, including tumour data from individual animals, the conclusions should be regarded as provisional.

6. SAFETY FOR THE CONSUMER

6.1. Metabolism and residues in the turkey

Radiolabelled (^{14}C at salicylic acid ring or the azomethine carbon) nifursol was administered into ligated loops of intestine (duodenum and upper jejunum) continuously for 8 hrs. Urine and bile were collected and analysed by thin-layer chromatography. The identity of the different metabolites (6) separated out was correctly determined using chemical degradation studies, infrared and ultraviolet spectrometry and gas-liquid chromatography [Lozano and Morrison, undated]

The metabolic pathway follows two routes. One is the hydrolysis of the azomethine bond followed by the oxidation of the furan moeity and conjugation to pyruvic acid and glucuronic acid of the other dinitrosalicylic moeity. The other consists of the reduction of the furan 5-nitro group followed with furan ring opening but keeping intact the azomethine bond and dinitrosalicylic structure.

It must be noted that a similar study conducted on the rat showed common metabolic pathways of nifursol in the turkey and rat, but also specific metabolites to each species [Lozano and Morrison, undated].

The results of analysis of bile were not reported. No identification of tissue residues was performed.

No investigation on the absorption, distribution and excretion of nifursol was conducted.

6.2. Residue depletion

No study was performed using repeted dosage with radiolabelled nifursol that would allow to establish the tissue residue steady state then depletion, target tissue and marker residue.

A residue study was performed in the practical conditions of use of nifursol. Eight-week-old turkeys were given feed containing a concentration of 75 mg/kg of nifursol for 8 weeks. Groups of 6 turkeys (sex not specified) were killed at the following times after the termination of dosing: 0, 1, 3, 5 and 7 days. Samples of liver, kidney, muscle and skin were frozen for later assay. The tissue samples were assayed by a method based on gas chromatography and electron capture detection which indirectly measured nifursol as its hydrolysis fragment 5-nitro-2-furaldehyde (which is not a nifursol metabolite). The method was validated in terms of nifursol recovery, and its limit of quantitation was 10 µg/kg. No nifursol residues were detected in any tissue at any withdrawal time. [George et al., 1973].

6.3. ADI

The conclusions drawn from the mutagenicity, genotoxicity and carcinogenicity studies, as well as the non-conclusive results of a chronic toxicity study in dogs [Rude, 1970a and 1970b] 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.

7. CONCLUSION

As both the ADI and the human exposure to nifursol residues (including metabolites) cannot be established, the safety of nifursol for the human consumer cannot be ensured.

8. REFERENCES

Allen JA and Proudlock RJ, 1987, "Micronucleus test on nifursol", Company report, HRC Report No. PDR 455/878, Huntingdon Research Centre, Huntingdon, England.

Allen JA, Proudlock RJ and Birt DM, 1987, "Analysis of metaphase chromosomes obtained from bone marrow of rats treated with nifursol", Company report, HRC Report No. PDR 458/87564, Huntingdon Research Centre, Huntingdon, England.

Benford DJ, 1987a, "Nifursol: Unscheduled DNA synthesis in hepatocytes and intestinal cells following oral exposure of rats", Report No. 10/87/TX, Robens Institute, University of Surrey, England.

Benford DJ, 1987b, "Intestinal irritation by nifursol", Report No. 18/87/TX, Robens Institute, University of Surrey, England.

Cavagnaro J and Cortina T, 1985a, "*In vitro* chromosomal aberrations in Chinese hamster ovary cells with nifursol", Company report, Hazleton Report No. 186-110.

Cavagnaro J and Cortina T, 1985b, "*In vitro* chromosomal aberrations in Chinese hamster ovary cells with nifursol (repeat test)", Company report, Hazleton Report No. 186-110.

Cavagnaro J and McCarrol NE, 1985, "*Salmonella typhimurium*/mammalian microsome plate incorporation assay with compound nifursol", Company report, Hazleton Report No. 186-107.

Cavagnaro J and Sernau RC, 1985, "Final report: unscheduled DNA synthesis rat hepatocyte assay", Company report, Hazleton Report No. 186-109.

Connelly J, 1988, "Investigation of binding of nifursol to rat tissue DNA *in vivo*", Report No. 11/87/TX, Robens Institute, University of Surrey, England.

Dawes RLF, 1988, "Nifursol - analysis of benign tumours", Company report, Internal Document No. 56645/86/88, Duphar B.V., Weesp, The Netherlands.

Frahm LJ, George GM and McDonnell JP, 1975, "Gas-liqid chromatographic determination of nifursol in frozen turkey tissues to ten parts per billion", Journal of the AOAC, **58**(4): 694 - 699.

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.

George GM, Frahm LJ and McDonnell JP, 1974, "The determination of nifursol /3,5-dintrosalicylic acid (5-nitrofurylidene) hydrazide/ in frozen turkey tissues to 10 ppb", Pharmaceutical Development and Analysis Department, Salisbury Laboratories, Charles City, Iowa. Research Technical Memorandum No. 162B. (Revised edition superseding the earlier version: Research Technical Memorandum No. 162)

Green SI, 1980, “Mutagenicity testing of nifursol using Ames’ test system as performed by M+E consultants, 35 Dean Hill, Plymouth PL9 9AF. Dated Jan 1980”, Company report, Wickam Laboratories, Wickham, Hants. PO17 5EU.

Jens DH, 1987, letter from Duphar, Weesp, 29 October 1987. Veterinary Products Committee paper SP3797.2 giving summaries of various studies.

Jorgenson TA, 1967, “Three generation reproduction study in rats”, Company report, Project No. 145, Test No. RRT-35-67, Salsbury Laboratories, Research Division, Charles City, Iowa.

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.

Narveson J, 1988, “To evaluate the effect of nifursol with respect to benign and malignant neoplasms (ie. Determine whether the tumor-incidence in treated animals was significantly different from non-treated controls)”, unpublished report of Salsbury Laboratories Inc, Iowa, USA.

Rude TA, 1970c, “Rat chronic toxicity: Chronic oral toxicity study of nifursol given continuously in the feed of rats for two years and 3 months (27 months)”, Company report, Project No. 145, Test No. RCT-35-67, Salsbury Laboratories, Research Division, Charles City, Iowa.

Rude TA, 1970a, “2 year study in dogs”, Company report, Project No. 145, Test No. DCT-35-67, Salsbury Laboratories, Research Division, Charles City, Iowa.

Rude TA, 1970b, “Pathology associated with a chronic oral toxicity test of nifursol given continuously in the feed of dogs for two years”, Company report, Project No. 145, Test No. DCT-35-67, Salsbury Laboratories, Research Division, Charles City, Iowa, USA.

van Kolfschoten, 1988, “Review of the genotoxic potential of nifursol”, Company report, Internal Document No. 56645/74/88, Duphar B.V., Weesp, The Netherlands.

Wood JD, Coleman M, Heywood R, Street AE, Gopinath C, Jolly DW, Gibson WA and Anderson A, 1984, “Nifursol toxicity to rats by continuous dietary administration for 13 weeks followed by a 4-week withdrawal period (final report)”, Company report, HRC Report number SLY 3/84961, Huntingdon Research Centre, Huntingdon, England.