Data Evaluation Report on the reproductive effects of BAS 510 F (TGAI) on avian species mallard duck, *(Anas platyrhynchos)*

PMRA Submission Number: 2001-1027 **EPA MRID Number: 454049-26**

Data Requirement: PMRA DATA CODE: 9.6.3.2
EPA DP Barcode: D278418
OECD Data Point: IIA 8.1.4
EPA Guideline: 71-4

Test material: **Purity (%):** 96.3
Common name: Nicobifen
Chemical name
IUPAC: 2-chloro-N-(4'-chlororobiphenyl-2-yl) nicotinamide
CAS name: 3-Pyridinecarboxamide, 2-chloro-N_(4'-chloro[1.1'-biphenyl]-2-yl)
CAS No.: 188425-85-6
Synonyms:

Primary Reviewer: Peter Takacs **Date:** October 8/02
{EPA/OECD/PMRA}

Secondary Reviewer(s): John Ravenscroft **Date:** November 22, 2002
{EPA/OECD/PMRA}

Company Code: BAZ
Active Code: CHH-BAZ-4
Use Site Category: In Canada, this fungicide is proposed for use on USC 13, 14 and 30; agricultural feed, food and turf uses. BAS 510 F is to be used 2-6 times per growing season depending on the crop, at a maximum recommended application rate of up to 875 g a.i./ha/application.
EPA PC Code: 128008

CITATION: S. Zok, November, 2000. BAS 510 F - l-Generation Reproduction Study On the Mallard duck *(Anas platyrhynchos)* by Administration in the Diet BASF Aktiengesellschaft Experimental Toxicology and Ecology 67056 LudwigshafedRhein, Germany, BASF study # 2000/1018527.

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EXECUTIVE SUMMARY:

The one generation reproductive toxicity of BAS 510 F to groups of 16 pairs of 180-day-old mallard ducks was assessed over 154 days in accordance with the Avian Reproduction Test, EPA 540/9-86- 139, July 1986 and OECD Guideline for Testing of Chemicals, 206 . BAS 510 F was administered to the birds in the diet at 0, 100, 300 and 1000 mg ai/kg dw diet. The NOEC (NOAEL) of BAS 510 F to the mallard, based on the reproductive parameters, was >1000 mg ai/kg dw diet, the highest test concentration used. No compound related mortality occurred in adult birds and no clinical signs of toxicity were noted. Statistically significant reductions in reproductive parameters did not occur at any concentration.
This toxicity study is classified as acceptable and satisfies the guideline requirement for a bobwhite quail reproductive toxicity study.

Results Synopsis

Test Organism Size/Age: 6-7 months old
NOEC:{>1000 mg a.i./kg dw diet}
LOEC:{>1000 mg a.i./kg dw diet}
Endpoint(s) Affected: None were significantly reduced compared to control.

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I. MATERIALS AND METHODS

GUIDELINE FOLLOWED:

(U.S.) EPA-FIFRA Protocol PB 83-153908 of October 1982, § 71-4 Standard Evaluation Procedure (= SEP), Avian Reproduction Test, EPA 540/9-86-139, July 1986.
OECD Guideline for Testing of Chemicals, 206 (adopted April 4, 1984).

COMPLIANCE:

This study was conducted in accordance with the GLP provisons of the Chemicals Act and with the OECD Principles of Good Laboratory Practice (Paris, 1981).

A. MATERIALS:

1. Test Material

Description: white powder
Lot No./Batch No. : N 46
Purity: 96.3%
Stability of Compound Under Test Conditions: stable for at least 10 days in feed
Storage Conditions of Test Chemicals: room temperature

Physicochemical properties of BAS 510 F.

Parameter	Values	Comments
Water solubility at 20°C	4.69 mg/L	low solubility
Vapour pressure	$7x10^{-9}$ mbar @ 20 °C	not volatile
UV absorption	UV molecular extinction: $1.53x10^{3}$ at 290 nm	-
pKa	does not dissociate in water	-
Kow	2.96	Not likely to bioconcentrate

2. Test organism:

Species (common and scientific names): mallard duck, *(Anas platyrhynchos)*
Age at study initiation: 6-7 months
Weight at study initiation: not stated

Source: John R.E. Coles, The County Game Farms, Home Farm, Ashford - Kent, TN26 1DR

B. STUDY DESIGN:

1. Experimental Conditions

b) Definitive Study

Table 1 . Experimental Parameters

Parameter	Details	Remarks *Criteria*
Acclimation Period: Conditions (same as test or not): Feeding: Health (any mortality observed):	3 weeks total 5 days in study pens, 15 days in communal area, under lab conditions. From arrival in the laboratory onward, "Ssniff', complete commercial diet for ducks in meal form without medical additives All birds were healthy, 4 additional pairs were used per treatment	acceptable *EPA recommends 2-3 week health observation period prior to selection of birds for treatment. Birds must be generally healthy without excess mortality. Sickness, injuries or mortality should be noted. Feeding should be ad libitum OECD requires acclimation of at least 2 weeks*
Test duration Pre-laying exposure: Egg-laying exposure: Withdrawal period, if used:	10 weeks each for pre- egg laying period and 12 weeks post.	acceptable

Parameter	Details	Remarks *Criteria*
		Pre-laying exposure duration *EPA /OECD require at least 10 weeks prior to the onset of egg-laying.* *Exposure duration with egg-laying* *EPA requires at least 10 weeks.* *Withdrawal period* *EPA requires if reduced reproduction is evident, a withdrawal period of up to 3 weeks should be added to the test phase.*
Pen (for parental and offspring) Size: Construction materials: Number:	stainless steel wire mesh cages, floor area: 1.3 x 0.65 m (about 0.85 m2 for 2 birds); height 1.3 m 16 cages per treatment + 3 extra cages of birds	acceptable *EPA requirements:* *Pens* *Adequate room and arranged to prevent cross contamination* *Materials* *Nontoxic material and nonbinding material, such as galvanized steel.* *Number* *At least 5 replicate pens are required for mallards housed in groups of 7. For other arrangements, at least 12 pens are required, but considerably more may be needed if birds are kept in pairs. Chicks are to be housed according to parental grouping.*
Number of birds per pen (male:female)	1 male, 1 female	acceptable *EPA requires one male and 1 female per pen. For bobwhite, 1 male and 2 females is acceptable. For mallard, 2 males and 5 females is acceptable.*
Number of pens per group/treatment Negative control: Treated:	16 pens + 3 additional pens 16 pens + 3 additional pens	acceptable *EPA/OECD require at least 12 pens, but considerably more if birds are kept in pairs. At least 16 is strongly recommended.*

Data Evaluation Report on the reproductive effects of BAS 510 F (TGAI) on avian species mallard duck, *(Anas platyrhynchos)*

PMRA Submission Number: 2001-1027 **EPA MRID Number: 454049-26**

Parameter	Details	Remarks *Criteria*
Test concentrations (mg ai/kg diet) Nominal: Measured:	0, 100, 300, 1000 mg ai/kg dw feed 90-101% of nominal in the diet	acceptable *EPA requires at least two concentrations other than the control; three or more are recommended. The highest test concentrations should show a significant effect or be at or above the actual or expected field residue level.* *OECD requires measured concentration in diet should be at least 80% of nominal*
EEC/maximum labeled field residue anticipated and source of information:	EEC in diet of mallard duck: 93 mg ai/kg dw as determined by EAD spread sheet "Workbook 2" based on maximum label rate of 476 g ai/ha x 6 applications; used on bulb vegetables. Label date: March 30/2001.	acceptable *EPA requires the highest test concentrations should show a significant effect or be at or above the actual or expected field residue level. The source [i.e., maximum label rate (in lb ai/A & ppm), label registration no., label date, and site should be cited]*
Solvent/vehicle, if used Type: Amount:	none used	acceptable *EPA /OECD require corn oil or other appropriate vehicle and not more than 2% of diet by weight*
Was detailed description and nutrient analysis of the basal diet provided (Yes/No)	yes	acceptable *EPA requires a commercial breeder feed (or its equivalent) that is appropriate for the test species.*
Preparation of test diet	the test chemical (no carrier) was mixed in a beaker with the diet weekly	 *A premix containing the test substance should be mechanically mixed with basal diet. If an evaporative vehicle is used, it must be completely evaporated prior to feeding.*

Data Evaluation Report on the reproductive effects of BAS 510 F (TGAI) on avian species mallard duck, *(Anas platyrhynchos)*

PMRA Submission Number: 2001-1027 **EPA MRID Number: 454049-26**

Parameter	Details	Remarks *Criteria*
Indicate whether stability and homogeneity of test material in diet determined (Yes/No)	yes	acceptable
Were concentrations in diet verified by chemical analysis (Yes/No)?	Yes	acceptable
Feeding and husbandry	food was available ad libitum	acceptable
Test conditions (pre-laying) Temperature: Relative humidity: Photoperiod:	 21 C ° ± 2 35-70% 7hrs light, 17 hrs dark for up to week 7 by week 10, photoperiod was 17 hrs light, 7 hrs dark 180 lux	**humidity ranged lower than recommended** *Temperature:* *EPA: about 21°C (70°F)* *OECD: 22 ± 5°C* *Relative humidity:* *EPA: about 55%* *OECD: 50-75%* *Lighting:* *EPA/OECD: first 8 weeks: 7 h per day* *Thereafter:* *EPA: 16-17 h per day.* *At least 6 footcandles at bird level* *OECD: 16-18 h per day*
Egg Collection and Incubation		
Egg collection and storage Collection interval: Storage temperature: Storage humidity: Storage period:	 daily during egg laying period 16 C 60-90% eggs were set into incubator weekly	acceptable *EPA requires eggs to be collected daily; egg storage temperature approximately 16°C (61°F); humidity approximately 65%. Collection interval: daily*
Were eggs candled for cracks prior to setting for incubation?	Yes	acceptable *EPA requires eggs to be candled on day 0*
Were eggs set weekly?	yes	acceptable
When candling was done for fertility?	Day 14 and 21 of incubation	acceptable

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Parameter	Details	Remarks *Criteria*
		EPA requires: *bobwhite: approx. day 11* *mallard: approx. day 14* *OECD requires: 6-11 day*
When the eggs were transferred to the hatcher?	23 days	acceptable *EPA requires:* *Bobwhite: day 21* *Mallard: day 23*
Hatching conditions Temperature: Humidity: Photoperiod:	37.7-37.9 C 56-78% not stated	**Humidity was lower than recommended** *Temperature:* *EPA requires: 39°C (102°F)* *OECD requires: 37°C* *Humidity* *EPA requires: 70%* *OECD requires: 70-85%*
Day the hatched eggs were removed and counted	day 28	acceptable *EPA requires Bobwhite: day 24* *Mallard: day 27*
Were egg shells washed and dried for at least 48 hrs before measuring?	Yes	acceptable
Egg shell thickness No. of eggs used: Intervals: Mode of measurement:	all eggs laid on first day of selected weeks every second week micrometer, at 4 points	acceptable *EPA requires newly hatched eggs be collected at least once every two weeks. Thickness of the shell plus membrane should be measured to the nearest 0.01 mm; 3 - 4 measurements per shell.*
Reference chemical, if used Name: Concentration tested:	not used	

2. Observations:

Table 2: Observations

Parameter	Details	Remarks / Criteria
Parameters measured		
Parental: (mortality, body weight, mean feed consumption) Egg collection and subsequent development: (no. of eggs laid, no. of eggs cracked, shell thickness, no. of eggs set, no. of viable embryos, no. of live 3 week embryos, no. hatched, no. of 14-day survivors, average weight of 14-d old survivors, mortality, gross pathology, others)	mortality, body weight, mean feed consumption, palatability, post mortem examination. All birds were inspected generally at least on workdays throughout the study period. General condition, signs of toxic effects and abnormal behavior were monitored. Egg data total eggs, weights, quality, shell thickness, embryo survival, infertile eggs, chicks hatched, mortality and body wt. Of chicks, 14 day survivors.	acceptable *OECD requires that the mortality in the controls is not exceed 10% at the end of the test. The average number of 14 day-old survivors per pen in controls at least 14 and 12 for mallard and bobwhite, respectively. OECD requires average egg shell thickness for control group 0.34 and 0.19 for mallard and bobwhite, respectively* *EPA requires:* *body weight should be recorded at test initiation and a biweekly intervals up to week eight or up to the onset of egg laying and at termination.* • *Eggs laid/pen* • *Eggs cracked/pen* • *Eggs set/pen* • *Viable embryos/pen* • *Live 3-week embryos/pen* • *Normal hatchlings/pen* • *14-day-old survivors/pen* • *Weights of 14-day-old survivors (mean per pen)* • *Egg shell thickness* • *Food consumption (mean per pen)* • *Initial and final body weight (mean per pen)*
Indicate if the test material was regurgitated	no regurgitation was noted, test material was retained by birds, group 2 birds consumed slightly more treated diet than other groups	

Parameter	Details	Remarks *Criteria*
Observation intervals (for various parameters)	weekly for most variables	acceptable *Body weights and food consumption must be measured at least biweekly.*
were raw data included?	Yes	

II. RESULTS AND DISCUSSION:

A. MORTALITY:

Parent generation: There was no mortality that could be attributed to the test compound. Only one bird died during the study, in group 1.

B. REPRODUCTIVE AND OTHER ENDPOINTS:

No compound-related effects in the parent generation on mortality, birds' health, palatability, feed consumption and body weight could be detected. Isolated incidence of lesions and aggressive behavior were noted, and such pairs were removed from the study.

Clinical observations
Clinical signs attributable to the test compound were not observed.

Feed consumption

The statistical analyses showed a statistically significant increase in the food consumption of group 2 during weeks 7 and 8 of the pre-egg laying period. The slight increase in the food uptake of group 2 during the pre-egg laying period was not reflected in a markedly higher body weight at day **56.**

Palatability
No rejection of feed containing the test compound could be observed.

Body weights
The body weight development in the male and female birds in the dose groups was not significantly different from the control group. The' statistical analyses revealed no evidence of any treatment effect for all days and both sexes.

Number of eggs laid
The mean number of eggs laid per female duck per week was used for the statistical evaluation. The statistical analysis revealed no evidence of any dose effect

Egg weights
The statistical evaluation (Dunnett's Test) revealed no evidence of any dose effect.

Egg quality
The statistical analyses (Wilcoxon-Test) revealed no evidence of any treatment effect.

Egg shell thickness
The statistical analysis revealed no evidence of any dose effect.

Fertility rate, infertile eggs
The overall fertility rates (% fertile eggs of eggs set) over the total egg-laying period were:
89.2% (group 0 = control)
94.3% (group 1 = 100 mg/kg)
86.3% (group 2 = 300 mg/kg)
92.1 % (group 3 = 1000 mg/kg diet)
For the proportion of fertile eggs of eggs initially set the Wilcoxon-test revealed no evidence of any dose effect

Early embryonic deaths and viable l4-day old embryos
The rates of early embryonic deaths of fertile eggs for the total period were:
3.6% (group 0 = control)
5.3% (group 1 = 100 mg/kg)
1.7% (group 2 = 300 mg/kg)
5.5% (group 3 = 1000 mg/kg diet)
The statistical analysis revealed no evidence of any dose effect.

The rates of viable 14-day embryos of eggs initially set for the total period were:
85.9% (group 0)
89.3% (group 1)
84.8% (group 2)
87.2% (group 3).

For the proportion of eggs set at day 14 (viable embryos) of eggs initially set the Wilcoxon-test revealed no evidence of any dose effect

Late embryonic deaths and live 18-day old embryos, percentage of fertile eggs

The rate of late embryonic deaths as percentage of fertile eggs was:
group 0 (control): 1 **.O%**

for group 1 (100 mg/kg diet): 0.7%
for group 2 (300 mg/kg diet): 0.5%
for group 3 (1000 mg/kg diet): 0.8%

For the proportion of late embryonic deaths of fertile eggs, proportion of eggs set at day 21 (live 21-day embryos) of fertile eggs, proportion of eggs set at day 21 (live 21-day embryos) of eggs initially set, and the proportion of eggs set at day 21 of eggs set at day 14, the Wilcoxon-test revealed no evidence of any statistically significant dose effect.

Total embryonic deaths, percentage of fertile eggs

Corresponding to the rates of early and late embryonic deaths the total embryonic deaths of fertile eggs for the total egg-laying period were:

for group 0 (control): 4.6%
for group 1 (I00 mg/kg diet): 6.0%
for group 2 (300 mg/kg diet): 2.2%
for group 3 (I000 mgkg diet): 6.3% (see Table 18).
These findings indicate no compound-related impairment of embryo survival in any of the dose groups in comparison to the control.

"Dead-in-shell" of fertile eggs
The statistical analyses (Wilcoxon-test) revealed no evidence of any statistically significant dose effect.

HATCHING RESULTS
For the number of hatched chicks per female duck and week the statistical analyses revealed a slight but statistically significant decrease in comparison to the control group in dose group 3 for weeks 9 - 12. Over the total study period the number of hatched chicks per hen and week significantly reduced and during the weeks 1 - 4 and 5 - 8 the number of hatched chicks per hen and week in the highest dose group was slightly higher than in the control group. The deviation seen in group 3 in comparison to the control group is not considered to be substance-related or biologically relevant.
The overall hatch rates (% hatched chicks of fertile eggs) over the total egg-laying period were:
72.2% (control)
63.9% (group 1 = 100 mgkg diet)
83.9% (group 2 = 300 mgkg diet)
71.8% (group 3 = 1000 mgkg diet)
For the proportion of hatched chicks of fertile eggs and of eggs set the Wilcoxon-test revealed no evidence of any dose effect.

F1-CHICKS

Clinical observations
No toxic signs and/or significant malformations exceeding the normal proportion were seen in the chicks.

14-day survivors

The rates of chicks surviving at 14 days as a percentage of chicks hatched were:
96.0% (control)
95.1 % (group 1)
98.3% (group 2)
98.0% (group 3).
No statistical evidence of a dose effect was seen.

Body weight

The mean body weight for the chicks for each dose group at hatching was, for the total period (weeks 1 - 12), 35.9 - 37.0 g, that of the 14-day old survivors 187.4 - 193.4 g. The statistical evaluation using Dunnett's test revealed no evidence of any dose effect. In conclusion, there was no effect on chick body weight at hatch and on the body weight of the chicks 14 days post hatch.

NOAEL:

In conclusion, under the conditions of this reproduction study in mallard duck with BAS 510 F the "no observed adverse effect level" (NOAEL) was >1000 mg ai/kg diet.

LOAEL:

The lowest observed adverse effect level (LOAEL) was >1000 mg/kg diet.

Table 4. Reproductive and other parameters - Results for each test group indicate the units of measurement.

Parameter	Control	100 mg/kg diet	300 mg/kg diet	1000 mg/kg diet	NOEC/ LOEC
Eggs laid/group	909	947	1024	889	NOEC: >1000 mg/kg
Eggs laid/hen/week	3.2 (week 1-4) 6.0 (week 5-8) 5.0 (week 9-12) 4.7 (mean)	3.2(week 1-4) 6.4(week 5-8) 5.2 (week 9-12) 4.9 (mean)	3.9 (week 1-4) 6.6 (week 5-8) 5.6 (week 9-12) 5.3 (mean)	2.8 (week 1-4) 6.4 (week 5-8) 4.7 (week 9-12) 4.6 (mean)	NOEC: >1000 mg/kg
Egg weights	60.2	61.8	60.1	60.5	NOEC: >1000 mg/kg
Eggs set	762	796	807	755	N/A
Eggs cracked	4.1	3.1	1.2	1.4	NOEC: 1000 mg/kg
Fertility rate of eggs set	89.2%	94.3%	86.3%	92.1%	NOEC: >1000 mg/kg
Shell thickness (mm ± SD)	0.39 (0.0125)	0.4 (0.0143)	0.39 (0.0133)	0.39 (0.0105)	NOEC: >1000 mg/kg
Viable 21 day embryos	726	750	789	709	NOEC: >1000 mg/kg
% 14 day survivors/hatched chicks	96%	95.1%	98.3%	98%	NOEC: >1000 mg/kg
No. of hatchlings/hen	2.88	2.75	3.53	2.76	NOEC: >1000 mg/kg
No. Of hatched chicks/group	552	527	677	533	NOEC: >1000 mg/kg
Hatchling weight (g)	36.3	37	35.9	36.6	NOEC: >1000 mg/kg

Parameter	Control	100 mg/kg diet	300 mg/kg diet	1000 mg/kg diet	NOEC/ LOEC
No. of 14-day old survivors	532	506	665	522	NOEC: >1000 mg/kg
14-day old survivors weight	187.4	193.4	191.5	191.3	NOEC: >1000 mg/kg
Mean food consumption (g/bird/day)	132.9	146.1	150.9	144.6	NOEC: >1000 mg/kg
Weight of females (parent) g/bird At test initiation: At onset of egg laying/other: day 56	 1046.9 1054.1	 1035.5 1079.5	 1035.7 1065.9	 1027.5 1060.7	NOEC: >1000 mg/kg
Weight of males (parent) g/bird At test initiation: at test termination:	 1099.3 1136.6	 1118.4 1185.4	 1096.5 1203.1	 1095.3 1157.0	NOEC: >1000 mg/kg

C. REPORTED STATISTICS:

The animals in the study were randomly assigned to 4 groups (0 = control group, 1 = 100 mg/kg, 2 = 300 mg/kg, 3 = 1,000 mg test compound/kg food). For the variables egg weight, egg shell thickness and chicks' body weight, a comparison of each dose group with the control group was carried out. These comparisons were performed simultaneously via Danaid's test for the hypothesis of equal means. If the results of this test were significant, labels (* for p 5 0.05, ** for p 5 0.01) were printed together with group means in the summary tables. The test was performed two-sided. For the body weight and food consumption of parent ducks a parametric one-way ana-lysis of variance was done via the F-test (ANOVA). If the resulting p-value was equal or less than 0.05, a comparison of each dose group with the control group was carried out. These comparisons were performed simultaneously via Danaid's test for the hypothesis of equal means. If results of this test were significant, labels (* for p f 0.05, ** p 5 0.01) were printed together with the group means in the summary tables. Both tests were performed two-sided. For the egg data, No. of dead-in-shell and No. of hatched chicks, a non-parametric analysis was carried out. A pair-wise comparison of each dose group with the control was performed via the Wilcoxon-test for the hypothesis of equal medians. If the results of this test were significant, labels (* for p 5 0.05, ** for p 5 0.01) were printed in the summary tables. The test was performed one-sided. The pen is the smallest unit for the statistical analyses; consequently these values are used to generate the means, medians and the variability of the data (exception: body weight of adult ducks, where the sample unit is the individual bird). The means in the summary tables of this appendix may differ slightly from the values reported in the tables of the main report, since these values are calculated *as* means of means of the pens. For the calculation of the variability either the standard deviation or the minimal and maximal values were used. If proportions were analyzed, sums of each pen were used in the numerator

and in the denominator. The number of data available for the statistical test is shown in the tables. During the egg production period this number is normally 16. Only if no observations for one or more pens are available, the number is less than 16. The statistical analyses were performed using the SAS'I-System. For the analyses of the body weight and of the food consumption of parent ducks the DATATOX FO-System was used. (* for p *5* 0.05, ** for p *5* 0.01) were printed in the summary tables. The test was performed one-sided. The pen is the smallest unit for the statistical analyses; consequently these values are used to generate the means, medians and the variability of the data (exception: body weight of adult quails, where the sample unit is the individual bird). For the calculation of the variability either the standard deviation or the minimal and maximal values were used.

D. VERIFICATION OF STATISTICAL RESULTS BY THE REVIEWER:

The conclusions of the study and statistical determinations are judged to be valid. Statistical analysis conducted by US. EPA using "chicks.sas".

NOEC: >1000 mg ai/kg diet (see above table for specific endpoints)
LOEC: >1000 mg ai/kg diet

E. STUDY DEFICIENCIES: The humidity during the study ranged lower than that recommended by EPA; (35-70% vs 50-75%) also during incubation on the eggs. This is considered to be a minor deficiency.

F. REVIEWER'S COMMENTS: The author concluded that the NOAEL and the LOAEL were both 1000 mg ai/kg diet. However, there were no significant test chemical related effects at any of the three concentrations, thus the NOAEL and the LOAEL were considered to by > 1000 mg ai/kg by the reviewer.

G. CONCLUSIONS: This reproduction study is acceptable. The test chemical BAS 510 F had no statistically significant adverse effects on any reproductive endpoints. There was no lethal or clinical toxicity noted in the parental generation.

NOEC:{>1000 mg a.i./kg diet}
LOEC:{>1000 mg a.i./kg diet}
Endpoint(s) Affected: None
Most Sensitive endpoint(s): N/A

III. REFERENCES:

Approved 04/01/01 C.K.