

CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA

Kresoxim-Methyl

Chemical Code 5451, Tolerance 52544

12/23/99

I. DATA GAP STATUS

| | |
|------------------------|--|
| Chronic toxicity, rat: | No data gap; possible adverse effect |
| Chronic toxicity, dog: | No data gap; no adverse effects |
| Oncogenicity, rat: | No data gap; possible adverse effects |
| Oncogenicity, mouse: | No data gap; no adverse effects |
| Reproduction, rat: | No data gap; no adverse effects |
| Teratology, rat: | No data gap; no adverse effects |
| Teratology, rabbit: | No data gap; no adverse effects |
| Gene mutation: | No data gap; no adverse effects |
| Chromosome effects: | No data gap; no adverse effects |
| DNA damage: | No data gap; no adverse effects |
| Neurotoxicity: | No data gap; no adverse effects |

Toxicology one-liners are attached.

All record numbers through 164400 were examined.

** indicates an acceptable study.

Bold face indicates a possible adverse effect.

indicates a study on file but not yet reviewed.

File name: T174234

Vidair, 12/23/99

II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

These pages contain summaries only. Individual worksheets may contain additional effects.

COMBINED, RAT

** 52544-014; 164378; "Chronic Toxicity Study with Reg. No. 242 009 in Wistar Rats Administration in the Diet for 24 Months" (Mellert, W., BASF Aktiengesellschaft, Dept. of Toxicology, D-67056 Ludwigshafen/Rhine, FRG, Project ID No. 70C0180/91007, 10/24/94). Test article Reg. No. 242 009 (Batch Nos. N27, N30, N36; purity 93.7-96.6%) was administered to 20 rats/sex/dose through their feed for 2 years at 0, 200, 800, 8000 or 16000 ppm. The mean daily intake of test article was, in ascending order of feed concentration: males 0/9/36/370/746 mg/kg; females 0/12/48/503/985 mg/kg. No treatment-related mortalities, abnormal clinical signs, or alterations in food consumption were observed. Mean bodyweights of treated males were consistently lower than controls for animals fed 8000 (5% lower) or 16000 (4% lower) ppm, and the difference was statistically significant at week 6. Females fed 8000 or 16000 ppm had significantly lower (average 8% lower; $p \leq .05$) mean bodyweights than controls. Analysis of blood from treated animals identified a single change considered to be directly caused by the test compound: that of increased (up to 49-fold, $p \leq .01$) γ -glutamyltransferase activity in males fed 8000 or 16000 ppm. At necropsy, absolute liver weights were 116% of control values ($p \leq .05$) for males fed 16000 ppm, while relative liver weights of males were 114% ($p \leq .05$) and 121% ($p \leq .01$) of controls for 8000 and 16000 ppm, respectively. Weights of female livers were not altered significantly by the test compound. Microscopic examination of livers from treated animals identified both neoplastic and non-neoplastic changes. The incidence of hepatocellular carcinoma was: males 0/1/1/3/8**; females 1/0/2/6/6; males + females 1/1/3/9*/14*** for 0/200/800/8000/16000 ppm, respectively (* $p \leq .05$, ** $p \leq .01$, *** $p \leq .001$). There were also 2 cholangiocarcinomas detected in males fed 200 ppm and one cholangioma in females fed 8000 ppm and in males fed 16000 ppm. Statistically significant increases in the incidence of the following non-neoplastic microscopic lesions in liver were measured: eosinophilic foci in males fed 8000 ppm ($p \leq .05$) or 16000 ppm ($p \leq .01$); cellular hypertrophy in males ($p \leq .05$) and females ($p \leq .05$) fed 16000 ppm. Mixed cell foci and biliary cysts were also increased in treated males, but the increases were not statistically significant. Macroscopic lesions of the liver were also increased in treated males (cysts) and females (masses). **Possible adverse effect:** increased incidence of hepatocellular carcinoma. **Chronic NOEL (M/F): 800 ppm (male 36 mg/kg/day; female 48 mg/kg/day)** (based upon increased relative liver weights, increased incidence of eosinophilic foci of the liver, and increased blood γ -glutamyltransferase activity for males, and decreased mean bodyweights for females, all in animals fed ≥ 8000 ppm). **Study acceptable** (Vidair 8/25/99).

CHRONIC TOXICITY, RAT

See combined toxicity, rat.

CHRONIC TOXICITY, DOG

** 52544-013; 164377; "Study of the Toxicity of Reg. No. 242 009 (BAS 490 F) in Dogs: Administration via the Diet Over 12 Months" (Hellwig, J., BASF Aktiengesellschaft, Dept. of Toxicology, D-67056 Ludwigshafen/Rhine, FRG, Project ID No. 33D0180/91090, 9/16/94). Test

article Reg. No. 242 009 (Batch No. N36 (=III C1); purity = 93.7%) was administered to 5 male and 5 female Beagle dogs/dose level in their feed for one year at 0, 1000, 5000 or 25000 ppm. Mean daily intake of the test article, in ascending order of feed concentration, was: males 0, 27, 138, 714 mg/kg; females 0, 30, 146, 761 mg/kg. The test compound caused no mortality or remarkable clinical signs other than sporadic vomiting and/or diarrhea in a few animals fed 25000 ppm. Food consumption was also normal. Mean bodyweights of males fed 25000 ppm were significantly ($p \leq 0.02$) lower than controls beginning at day 182 and continuing to the study's end (except for a single week), averaging 8% lower at day 182 and 11% lower at day 364. Bodyweights of females were not affected. Measurements of blood and urine composition were normal except for significant elevations of platelet counts in males only, observed at all 3 time points (days 93, 181, 363). Increased relative liver weights in males fed 5000 ppm (increased 9%, $p \leq 0.05$) or 25000 ppm (increased 17%, $p > 0.05$) were the only abnormalities at necropsy. No cellular basis for these liver effects could be identified by histopathology, casting doubt on their toxicological significance. **No adverse effects indicated. NOEL (M) = 5000 ppm** (138 mg/kg, based upon decreased mean bodyweights for males fed 25000 ppm), **(F) = 25000 ppm** (761 mg/kg, based on the absence of toxicological effects in females fed 25000 ppm). **Study Acceptable** (Vidair 8/19/99).

ONCOGENICITY, RAT

52544-011; 164361; "Statement Concerning the Determination of Cell Proliferation in the Liver of BAS 490 F Treated Rats" (van Ravenzwaay, B., BASF Aktiengesellschaft, Dept. of Toxicology, D-67056 Ludwigshafen/Rhine, FRG, BASF Registration Document No. 97/10286, 3/31/97). Five male rats per dose of BAS 490 F were offered food containing the test article at 0 or 16000 ppm for 1, 6 or 13 weeks, with or without a recovery period of 2-5 weeks after test compound withdrawal. One week prior to sacrifice, osmotic minipumps were implanted so as to provide perfusion of BrdU and thus allow identification of proliferating cells. The test article at 16000 ppm induced significant increases in hepatocyte labeling indices of 71% after 1 week of feeding, 49% after 6 weeks, and 37% after 13 weeks. Only the increases after 1 and 6 weeks were significant relative to controls, suggesting an adaptive response of the liver cells with increasing time of exposure. The increases in labeling indices were greatest in the zone 1 cells compared to zones 2 and 3. Furthermore, these effects were reversible, with labeling indices dropping to below control values 2-5 weeks after test compound removal. When the data from a number of S phase response studies were tabulated, a dose-response was demonstrated for this endpoint, with liver cell proliferation exhibiting stimulation by 8000 ppm and above, but not by 800 ppm and below. **NOEL (M) = 800 ppm** (based upon an increased labeling index for hepatocytes in animals fed 8000 ppm). **Supplemental Study** (Vidair 8/18/99).

52544-011; 164362; "Expert Report on the Evaluation of Hepatic Cell Proliferation in Liver from Rats Exposed to BAS 490 F" (Swenberg, J. and Goldsworthy, T., BASF Aktiengesellschaft, Dept. of Toxicology, D-67056 Ludwigshafen/Rhine, FRG, BASF Registration Document No. 96/11092, 12/9/96). The authors were asked to review the studies showing that the test article induced the proliferation of liver cells in rats fed ≥ 8000 ppm. The reviewers confirmed that the methodology used, that of BrdU infusion in live animals followed by measurement of labeling indices, was the method of choice for these studies. After reviewing the data they concluded the following: 1) the test article stimulated liver cell proliferation only at dose levels which also induced tumors 2) the stimulation of proliferation was reversible 3) stimulation was greatest after 3

weeks of compound administration 4) zone 1 of the liver (periportal region) was the most sensitive. They hypothesized that the test article stimulated the proliferation of already initiated cells, thereby acting as a tumor promoter. **Supplemental study** (Vidair 9/30/99).

52544-015; 164379; "Pathology Working Group (PWG) Report on Compound Reg. No. 242 009; 24-Month Chronic Toxicity (Project No. 70C0180/91006) in Wistar Rats" (Hildebrandt, P., PATHCO, Inc., Ijamville, MD, Doc. No. 95/10977, 8/9/95). This was a review of the pathology data from a 2 year Oncogenicity/Carcinogenicity study in rats performed at BASF Corporation (Record #164381). A panel of 7 pathologists reviewed slides of liver sections from animals in that study. The panel examined all primary neoplasms as well as livers from animals with no neoplasms. **Possible adverse effects:** the consensus was that the test compound caused an increased incidence of hepatocellular neoplasms in male and female rats fed 8000 or 16000 ppm relative to controls. The increases were statistically significant (in the Fisher's Exact Test conducted by investigators) for hepatocellular carcinoma in males fed 8000 ppm ($p \leq .027$) and females fed 8000 ppm ($p \leq .03$) or 16000 ppm ($p \leq .03$), and for hepatocellular adenoma in females fed 8000 ppm ($p = 0.0$) or 16000 ppm ($p = 0.0$). **Supplemental study** (Vidair 8/30/99).

52544-015; 164380; "Pathology Working Group (PWG) Report on Compound Reg. No. 242 009; 24-Month Chronic Toxicity (Project No. 70C0180/91007) in Wistar Rats" (Hildebrandt, P., PATHCO, Inc., Ijamville, MD, Doc. No. 95/10979, 8/9/95). This was a review of the pathology data from a 2 year Subchronic Oral Toxicity study in rats performed at BASF Corporation (Record #164378). A panel of 7 pathologists reviewed slides of liver sections from animals in that study. The panel examined all primary neoplasms as well as livers from animals with no neoplasms. **Possible adverse effects:** the consensus was that the test compound caused an increased incidence of hepatocellular neoplasms in male and female rats fed 8000 or 16000 ppm relative to controls. The increases were statistically significant (in the Fisher's Exact Test conducted by the investigators) for hepatocellular tumors (carcinomas + adenomas) in males fed 8000 ppm ($p \leq .053$) or 16000 ppm ($p \leq .004$) and females fed 8000 ppm ($p \leq .024$) or 16000 ppm ($p \leq .01$), and for hepatocellular adenoma in females fed 8000 ppm ($p \leq .053$) or 16000 ppm ($p \leq .053$). **Study supplemental** (Vidair 8/30/99).

** 52544-016; 164381; "Carcinogenicity Study with Reg. No. 242 009 (BAS 490 F) in Rats: Administration in the Diet for 24 Months" (Mellert, W., BASF Aktiengesellschaft, Dept. of Toxicology, D-67056 Ludwigshafen/Rhine, FRG, Project ID No. 70C0180/91006, 10/24/94). Test article Reg. No. 242 009 (Batch Nos. N27, N30, N36; purity 93.7-96.6%) was administered to 50 Wistar rats/sex/dose through their feed for 2 years at 0, 200, 800, 8000, or 16000 ppm. The mean daily intake of test article was: males 0/9/36/375/770 and females 0/12/47/497/1046 mg/kg for 0/200/800/8000/16000 ppm, respectively. Mortality, food consumption and food efficiency were unaffected by the test article. In addition, clinical signs and differential blood counts were similar in treated and control animals. Bodyweights were significantly lower for both sexes fed the two highest dose levels: at 8000 ppm for at least 30/36 time points and at 16000 ppm for the entire period of test article administration. Males fed 8000 or 16000 ppm weighed approximately 10% less than controls by study's end ($p \leq .05$), while females fed 8000 or 16000 ppm weighed approximately 12% or 14% less than controls ($p \leq .01$) by study's end, respectively. Absolute kidney weights for males fed 8000 or 16000 ppm, and for females fed 8000 ppm, were about 7% lower than controls ($p \leq .05$). In contrast, relative brain weights for males fed 8000 ppm ($p \leq .05$) and for females fed 8000 or 16000 ppm ($p \leq .01$) were from 12-15% higher than controls. There were no obvious histological correlates to explain these effects on the kidneys or brain. Relative liver weights of females fed the highest dose were approximately 10% higher than controls ($p \leq .01$). Correspondingly, both gross pathological and histopathological alterations were identified in livers of treated animals. Treated males and females had increased numbers of liver cysts and masses.

At the cellular level, treated males had an increased incidence of eosinophilic foci, biliary cysts, mixed cell foci and cellular hypertrophy relative to controls, while treated females exhibited increases in cholangiofibrosis, mixed cell foci, altered cell foci, and bile duct proliferation relative to controls. Hepatocellular carcinomas were also increased in treated animals: males 7/5/2/18*/11 and females 1/1/2/13***/16*** for 0/200/800/8000/16000 ppm, respectively (* $p \leq .05$, *** $p \leq .001$). Hemangiosarcoma of the spleen was increased by treatment in both sexes: males 0/0/2/2/3 and females 0/1/0/1/1 for 0/200/800/8000/16000 ppm, respectively. The number of tumor bearing animals and the total number of primary neoplasms were not markedly affected by the test compound, and none of the animal deaths occurring during the study were judged to have been caused by liver cancer. **Possible adverse effect:** increased incidence of hepatocellular carcinoma. **NOEL (M/F) 800 ppm (male 36 mg/kg/day; female 47 mg/kg/day)** (based upon decreased mean bodyweights and increased incidence of gross pathological and microscopic alterations to the livers of animals in the 8000 ppm and 16000 ppm treatment group). **Study acceptable** as an oncogenicity study only (no clinical chemistry, urinalysis or ophthalmology) (Vidair 8/27/99).

52544-018; 164383; "Pathology Working Group (PWG) Report on Compound Reg. No. 242 009; 24-Month Chronic Toxicity (Project No. 70C0180/91007) and Carcinogenicity (Project No. 70C0180/91006) in Wistar Rats" (Hildebrandt, P., PATHCO, Inc., Ijamville, MD, Doc. No. 95/10980, 8/9/95). This was a review of the data from a pair of 2 year feeding studies in the rat: Chronic Toxicity Study Record #164378 and Oncogenicity/Carcinogenicity Study Record #164381. In this review the data from the two studies were combined and analyzed by various statistical methods, including the Fisher's Exact Test. **Possible adverse effects:** hepatocellular tumors (adenomas plus carcinomas) were significantly increased by the test compound in females fed 8000 ppm (21/70 vs. 1/70, $p=0.0$) or 16000 ppm (23/70 vs. 1/70, $p=0.0$) and in males fed 8000 ppm (20/70 vs. 6/70, $p \leq .002$) or 16000 (18/70 vs. 6/70, $p \leq .006$), all relative to controls. Importantly, combining these two studies revealed significant increases in the incidence of neoplasms in females fed 800 ppm; hepatocellular adenomas were increased (5/70 vs. 0/70, $p \leq .029$) as were hepatocellular tumors (7/70 vs. 1/70 $p \leq .031$), both relative to controls. Lastly, it was noted that the 8000 and 16000 ppm treatment groups each included 4 animals with multiple tumors, whereas no animals with multiple tumors occurred at lower doses or among controls. **Supplemental study** (Vidair 9/1/99).

52544-018; 164384; "Assessment of the Mechanism and Dose Response Relationship of the Potential Carcinogenicity of Kresoxim-methyl" (van Ravenzwaay, B., BASF Aktiengesellschaft, Dept. of Toxicology, D-67056 Ludwigshafen/Rhine, FRG, BASF Registration Document No. 98/10207, 2/25/98). This report addresses whether Kresoxim-methyl should be classified as a human carcinogen. It clearly caused an increased incidence of hepatocellular carcinoma in rats fed 8000 and 16000 ppm over two years. However, the author argues that this should not lead to its designation as a human carcinogen for three reasons. First, the test article did not cause cancer in mice. Second, the test article probably acts as a tumor promoter by stimulating cell growth, as exemplified by its ability to stimulate the proliferation of liver cells at 8000 and 16000 ppm. Since growth stimulation of mammalian cells exhibits a threshold response to growth-promoting stimuli, it follows that doses below the threshold would be ineffective. Lastly, it is argued that potential human exposure would be transient, and at levels well below those of test animal exposure. Since the stimulation of liver cell proliferation by the test article was shown to be reversible, transient exposure may not induce cell proliferation for a period of time sufficient to promote tumor formation. **Study supplemental** (Vidair 9/28/99).

52544-018; 164385; "Assessment of the Mode of Action of Kresoxim-methyl as a Non

Genotoxic Carcinogen in Rats” (van Ravenzwaay, B., BASF Aktiengesellschaft, Dept. of Toxicology, D-67056 Ludwigshafen/Rhine, FRG, BASF Registration Document No. 98/10253, 3/12/98). This report discusses the carcinogenicity of Kresoxim-methyl in laboratory animals, its mode of action, and the potential risk it poses as a human carcinogen. In rats fed 8000 or 16000 ppm for 2 years there was an increased incidence of hepatocellular carcinoma. No effect was observed at 800 ppm. A pathology working group re-evaluated the liver pathology for that study, along with a smaller chronic study in the rat. When data from the 2 studies were combined (Record #164383), there was a significant increase in adenomas ($p \leq 0.029$) for females fed 800 ppm. The author of the instant report argues that this increase at 800 ppm (7.1% incidence) was not indicative of a test article-induced effect because it was not significantly different from the incidence of adenomas in females from among all historical controls (2.7%), and was less than the maximum incidence of 20% for individual studies. As to the test article’s mode of action, the author cites the studies which show it is not genotoxic, either *in vivo* or *in vitro*. He describes an *in vivo* initiation study in which the test article was negative. In contrast, in an *in vivo* promotion study, animals fed the test article at 8000 and 16000 ppm had significant increases in liver foci. No increases were seen at 800 or 200 ppm. These data indicate that the test article is a promoter of liver foci in rats at ≥ 8000 ppm. Tumor promoters often act by stimulating cell proliferation. The author cites S phase studies which show that the test article stimulates liver cell proliferation in rats fed 16000 ppm, but not in animals fed 800 ppm. This hypothesis of increased liver cell proliferation as the mode of action is supported by observations from the 2 year carcinogenicity study, which included liver enlargement, liver cell hypertrophy, bile duct proliferation and increased gamma-glutamyltransferase. These effects were observed only at ≥ 8000 ppm. A scenario is offered whereby the test article acts on already initiated cells to stimulate their proliferation, thus promoting tumor formation. This could explain the late occurrence of tumors in the 2 year oncogenicity study. The author believes the test article poses almost no risk as a human carcinogen for 2 reasons: 1) such high doses are required, 2) tumor induction probably requires continuous long-term exposure, whereas short term exposure would not suffice because the stimulation of liver cell proliferation is reversible. **Supplemental** (Vidair 9/29/99).

52544-049; 169458; “BAS 490 F (Reg. No. 242 009): Medium Term Promotion Hepatocarcinogenesis Study in Rats” (Harada, T., Institute of Environmental Toxicology, 2-772, Suzuki-cho, Kodaira-shi, Tokyo 187, Japan, Project Study No. IET 96-0098, 3/26/97). Two weeks after receiving an intraperitoneal injection of the initiator diethylnitrosamine (200 mg/kg), 16 male Fischer rats/dose level were administered Reg. No. 242 009 (Lot No. N112, 95.4% purity) in their feed for 6 weeks at 0, 200, 800, 8000, and 16000 ppm or the positive control phenobarbital at 500 ppm. At week 3 into the study each animal was given a 2/3 partial hepatectomy. After the 8 week period of test article administration, animals were sacrificed and necropsied, livers were weighed, and glutathione S-transferase placental form (GST-P) positive hepatic foci were measured. Mean daily test article intake was 10.72/42.47/430.6/886/27.99 mg/kg/day for 200/800/8000/16000/phenobarbital 500 ppm. There were no effects of test article administration on food consumption or bodyweights, although both parameters were significantly higher than negative controls ($p < .05$) in animals fed phenobarbital. Enlarged livers were observed in all animals fed 8000 (14/14) and 16000 (14/14) ppm of the test article or fed phenobarbital (14/14), and in 2/14 animals fed 800 ppm. Animals fed the test article or phenobarbital had mean absolute and relative liver weights which were higher than the negative controls, however, the increases were only significant ($p < .01$) for 800 ppm and above and for the positive control. Animals fed the test article or phenobarbital also had increased mean numbers and sizes of GST-P positive hepatic

foci relative to controls, however, the increases were only significant ($p < .01$) for 8000 ppm and above and for the positive control. Due to this induction of hepatocellular foci, the test article was considered a tumor promoter at 8000 and 16000 ppm. **Potential adverse effect:** tumor promotion at ≥ 8000 ppm. **Supplemental study** (Vidair 10/5/99).

ONCOGENICITY, MOUSE

** 52544-017; 164382; "Carcinogenicity Study with Reg. No. 242 009 (BAS 490 F) in Mice: Administration in the Diet for 18 Months" (Mellert, W., BASF Aktiengesellschaft, Dept. of Toxicology, D-67056 Ludwigshafen/Rhine, FRG, Project ID No. 65C0180/91028, 10/17/94). Test article Reg. No. 242 009 (Batch Nos. N30, N36; purity 93.7-96.6%) was administered to a main group of 50 mice/sex/dose through their feed for 18 months at 0, 400, 2000 or 8000 ppm. A satellite group of 10 mice/sex/dose received the test article for 12 months. The mean daily intake of test article for the main group was: males 0/60/304/1305 mg/kg and females 0/81/400/1662 mg/kg for 0/400/2000/8000 ppm, respectively. Mortality, food consumption and food efficiency were unaffected by treatment. In addition, there were no remarkable clinical signs specific to the treated groups, and differential blood counts from treated and control animals were similar. Mean bodyweights of main group animals were reduced by the test compound. At 8000 ppm males were significantly lower than controls for 11/30 time points (average 7.1% lower, $p \leq .05$), ending the study 9% lower than controls. Females fed the high dose had significantly lower mean bodyweights than controls for 9/30 time points (average 11.7% lower, $p \leq .01$), finishing the study 20% lower than controls. Females fed 2000 ppm were significantly lower than controls for 5/30 time points (average 8.8% lower, $p \leq .05$), ending the study 11% lower than controls. Among animals of the satellite group necropsied at 12 months, males fed 8000 ppm had larger adrenal glands: 35% heavier than controls for the absolute organ weight (not significantly different from controls) and 46% heavier than controls for the relative organ weight ($p \leq .05$). No histological findings were made to explain these changes, suggesting they were not compound-related. Main group animals necropsied at 78 weeks exhibited no remarkable changes in absolute organ weights, suggesting that the statistically significant increases in relative organ weights for males (kidneys, testes, adrenals) and females (liver, brain, adrenals) fed 8000 ppm were due to decreased bodyweights. The absence of histological correlates to the increased relative organ weights supports this explanation. The test compound did not increase the incidence of any neoplasm in either the main or satellite groups. However, a few non-neoplastic lesions were increased. Relative to controls, females fed 8000 ppm exhibited an increased incidence of kidney retraction and/or papillary necrosis of the kidneys, increased amyloidosis of the liver (both its incidence and severity), and increased fatty infiltration of the centrolobular region of the liver. Males fed 8000 ppm exhibited only increased inflammation of the preputial glands. **No adverse effects indicated. NOEL (F) 400 ppm** (81 mg/kg; based upon decreased mean bodyweights in females fed 2000 and 8000 ppm and increased incidence of amyloidosis of the liver, increased fatty infiltration of the liver, increased retraction of the kidneys, and increased papillary necrosis of the kidneys in females fed 8000 ppm); **(M) 2000 ppm** (304 mg/kg; based on reduced mean bodyweights in animals fed 8000 ppm). Study **acceptable** (Vidair 8/31/99).

REPRODUCTION, RAT

** 52544-021; 164388; "Reproduction Toxicity Study with Reg. No. 242 009 (BAS 490 F) in Rats: Continuous Dietary Administration Over 2 Generations" (Hellwig, J., BASF

Aktiengesellschaft, Dept. of Toxicology, D-67056 Ludwigshafen/Rhine, FRG, Project ID No. 70R0180/91093, 10/28/94). Twenty five rats /sex/dose were offered feed containing test article Reg. No. 242 009 (Batch No. N36 (IIIc₁); purity \geq 93.7%) at 0, 50, 1000, 4000 or 16000 ppm over two generations, called F0 and F1. F0 parents received the test article for at least 10 weeks before mating, throughout mating, and until necropsy during week 32. The F0 parents were bred twice during this time, producing two litters called F1a and F1b. These offspring were exposed to the test article in utero and during nursing, through lactation day 21, at which time the nonselected pups were subjected to necropsy. Twenty five male and female F1a offspring were selected (prior to weaning) from each treatment group, thus comprising the F1 parents. These animals were fed the test article for at least 98 days prior to mating, after which the F1 females produced F2 litters, which were treated as described above for the F1 litters. No mortality was induced by the test compound. Parental bodyweights of treated males and females(4000, 16000 ppm) from both generations were significantly lower than controls ($p < .05$). Mean food consumption was only sporadically (though significantly) lower in the treated animals, and could not account for the lower bodyweights. Another indicator of parental toxicity was the increase in serum γ -glutamyltransferase activity in males fed 4000 or 16000 ppm ($p < .01$) and in females fed 16000 ppm ($p < .05$). Since histopathology did not detect damage to hepatocytes that could have resulted in release of liver enzymes into the blood, it was suggested that the test compound induced the production of this enzyme. The relative weights of all organs measured (kidney, testes, liver, epididymides) were significantly increased relative to controls in F0 males fed 16000 ppm ($p < .01$). However, since the absolute organ weights of treated males were not significantly greater than controls, it was suggested that these increases in relative organ weight, as well as other increases in F1 males and F0 females, were due to lower bodyweights. All parental parameters of reproduction were normal. While pups born from females fed 4000 or 16000 ppm had normal bodyweights at birth, they fell significantly behind controls during lactation. Another developmental defect was noted: that of significantly ($p < .05$) decreased pinna unfolding by day 4 post partum in F1b pups from mothers fed 4000 or 16000 ppm. **No adverse effects indicated. Parental NOEL (M/F): 1000 ppm** (based upon lower mean bodyweights for F0 and F1 parents fed 4000 ppm and upon increased γ -glutamyltransferase levels in the blood of males fed 4000 ppm). **Reproductive NOEL (M/F): 16000 ppm** (based upon normal parameters of reproduction in both male and female parents fed 16000 ppm). **Developmental NOEL: 1000 ppm** (based upon lower mean bodyweights for the F1a, F1b and F2 offspring and upon decreased pinna unfolding in F1b pups, all in animals from the 4000 ppm treatment group). **Study acceptable** (Vidair 9/8/99).

TERATOLOGY, RAT

**52544-019; 164386; "Study of the Prenatal Toxicity of Reg. No. 242 009 in Wistar Rats After Oral Administration (Gavage)" (Hellwig, J., BASF Aktiengesellschaft, Dept. of Toxicology, D-67056 Ludwigshafen/Rhein, FRG, Project ID No. 30R0180/91122, 9/20/94). Test article Reg. No. 242 009 (Batch No. N36 (III c₁); purity \geq 93.7%) was administered to 25 mated female rats/dose at 0, 100, 400, or 1000 mg/kg/day. Administration was once daily by oral gavage, on days 6 through 15 post-coitum. The test article had no effect on maternal mortality, food consumption, bodyweight, or uterus weight, and produced no clinical signs. Small increases in postimplantation loss, early resorption and total resorption were noted in treated animals relative to controls; however, the increases were not statistically significant, no clear dose-responses were indicated, and the values for the treated animals fell within the ranges of historical controls. The only statistically significant effect on fetal development was an increased number of litters with

incompletely ossified thoracic vertebral bodies in the highest dose group (5/9/7/14*, corresponding to 0/100/400/1000 mg/kg, * $p \leq 0.05$). This finding was not considered of toxicological importance since this frequency (56% of litters) fell within the range for historical controls and the fetal incidence of retardations was not significantly altered by the test compound (78/83/75/98 for 0/100/400/1000 mg/kg). **No adverse effects indicated. Maternal NOEL = 1000 mg/kg/day** (based upon the absence of effects on dams fed 1000 mg/kg/day). **Developmental NOEL = 1000 mg/kg/day** (based upon the absence of effects on fetuses in the 1000 mg/kg/day group). **Study acceptable** (Vidair 9/1/99).

TERATOLOGY, RABBIT

** 52544-020; 164387; "Study of the Prenatal Toxicity of Reg. No. 242 009 (BAS 490 F) in Rabbits After Oral Administration (Gavage)" (Hellwig, J., BASF Aktiengesellschaft, Dept. of Toxicology, D-67056 Ludwigshafen/Rhine, FRG, Project ID No. 40R0180/91044, 7/29/93). Test article Reg. No. 242 009 (Batch No. N30 (IIIa₂); purity = 96.6%) was administered to 15 artificially inseminated female Himalayan rabbits/dose at 0, 100, 400 or 1000 mg/kg/day. Administration was once daily by oral gavage, on days 7-19 post-insemination. Dams were unaffected by the test compound in terms of mortality, bodyweight, uterus weight, clinical signs, and litter data. Food consumption increased in treated animals ($p < 0.01$). Fetuses from dams fed 100 mg/kg had an incidence of fused sternbrae which was significantly higher than controls (3/11*/7/9 for 0/100/400/1000 mg/kg, * $p < 0.05$). However, since the two higher doses gave values that fell within the ranges of historical controls, and there was no dose-response, the increase at 100 mg/kg was not considered to have been induced by the test compound. Fetal soft tissue malformations were only seen in treated animals, the total incidence being 0/2/2/4 for 0/100/400/1000 mg/kg. However, as noted above for the skeletal variation, since the numbers of fetuses with each individual type of soft tissue malformation (hydrocephaly, dilatation of the aortic arch and descending aorta, septal defect, agnesia of the gall bladder) were very low and within the ranges of historical controls, they were judged unrelated to treatment. **No adverse effects indicated. Maternal NOEL = 1000 mg/kg/day** (based upon the absence of effects on dams fed 1000 mg/kg/day). **Developmental NOEL = 1000 mg/kg/day** (based on the absence of effects on fetuses in the 1000 mg/kg/day group). **Study acceptable** (Vidair 9/2/299).

GENE MUTATION

** 52544-022; 164389; "Study of Reg. No. 242 009 (BAS 490 F) in the Ames Assay and E. Coli Reverse Mutation Assay" (Engelhardt, G., BASF Aktiengesellschaft, Dept. of Toxicology, D-67056 Ludwigshafen/Rhine, FRG, Project ID No. 40M0180/914324, 3/11/93). *S. typhimurium* tester strains TA1535 and TA100 (reverted to histidine autotrophy by base substitution), strains TA1537 and TA98 (reverted by frameshift mutations), and *E. coli* tester strain WP2 uvrA (reverted to tryptophan autotrophy by base substitution) were exposed to test article Reg. No. 242 009 (Batch # N27(=IIIaI); 93.7% purity) at 0 (vehicle only), 20, 100, 500, 2500 or 5000 ug/plate, with or without activation by an S9 microsomal fraction. Exposure was at 37°C for 48 hours, with or without an additional 20 min 37°C pre-incubation period (in which the bacteria, S9 fraction and test article were incubated together prior to the addition of agar). Each condition was tested once and assayed in three replicate plates. Positive controls were functional. The test article had no effect on the reversion frequency. There was no bacterial toxicity by the highest doses tested,

despite the formation of precipitate at 500 ug/plate and above. **No adverse effects indicated. Study acceptable** (Vidair 9/13/99).

** 52544-022; 164390; "Gene Mutation Test in Chinese Hamster Ovary Cells with Reg. No. 242 009 (BAS 490 F) (HPRT Locus Assay)" (Polloth, C., BASF Aktiengesellschaft, Dept. of Toxicology, D-67056 Ludwigshafen/Rhine, FRG, Project ID No. 50M0180/914335, 5/13/94). Test article Reg. No. 242 009 (Batch No. N36(IIIc1); 94.3% purity) was tested for its ability to induce mutations which inactivate hypoxanthine-guanine phosphoribosyl transferase (HPRT) in Chinese hamster ovary (CHO) cells. Cells were exposed to concentrations of up to 0.1 mg/ml for 4 hours at 37°C (precipitation began at ≥ 0.0215 mg/ml), followed by one week in normal medium. The frequency of HPRT inactivation was measured by testing the cells for colony formation in 6-thioguanine. Two independent, replicate experiments were performed, each with and without addition of an S9 microsomal fraction. Positive controls were functional. The test article did not affect the frequency of HPRT inactivation, indicating that it was not mutagenic in the assay. **No adverse effects indicated. Study acceptable** (Vidair 9/14/99).

** 52544-022; 164393; "Report on the Study of Reg. No. 242 009 in the Ames Test (Salmonella/Mammalian-Microsome Mutagenicity Test-Standard Plate Test and Preincubation Test). (Engelhardt, G., BASF Aktiengesellschaft, Dept. of Toxicology, D-67056 Ludwigshafen/Rhine, FRG, Project ID No. 40M0180/914342, 9/5/94); *S. typhimurium* tester strains TA1535, TA100 (both reverted to histidine autotrophy by base substitutions), TA1537 and TA98 (both reverted by frameshift mutations) were exposed to Reg. No. 242 009 (Batch No. N36 (=III c 1); 94.3% purity) at 0 (vehicle only), 20, 100, 500, 2500 and 5000 ug/plate, with or without activation by an S9 microsomal fraction. Exposure was at 37°C for 48 hours, with or without a 20 min 37°C pre-incubation (where the bacteria, test article and S9 fraction were incubated together prior to the addition of agar). Each condition was tested once and assayed in three replicate plates. Positive controls were functional. The test article had no effect on the reversion frequency, either in the presence or absence of an S9 fraction. Some bacterial toxicity, as well as test article precipitation, were noted for doses ≥ 2500 ug/plate. **No adverse effects indicated. Study acceptable** (Vidair 9/16/99).

52544-050; 169459; "Report on the Study of Reg. No. 291 685 in the Ames Salmonella/Mammalian Microsome Mutagenicity Test and Escherichia coli/Mammalian Microsome Reverse Mutation Assay, Test Substance: Plant Metabolite [BAS 490-2] of Kresoxim-methyl" (Hoffmann, H.D. and Engelhardt, G., BASF Aktiengesellschaft, Dept. of Toxicology, D-67056 Ludwigshafen/Rhein, Germany, Project ID No. 40M0262/944203, 12/30/94). *S. typhimurium* tester strains TA1535 and TA100 (reverted to histidine autotrophy by base substitution), strains TA1537 and TA98 (reverted by frameshift mutation), and *E. coli* tester strain WP2uvrA (reverted to tryptophan autotrophy by base substitution) were exposed to test article Reg. No. 291 685 (Batch No. 00436-65, 97.7% purity, plant metabolite BAS 490-2) at 0 (vehicle only), 4, 20, 100, 500, 2500 and 5000 ug/plate, with or without activation by an S-9 microsomal fraction. Exposure was at 37°C for 48 hours, with or without an additional 20 min 37°C pre-incubation period (in which the bacteria, S-9 fraction and test article were incubated together prior to the addition of agar). Each condition was tested once in each tester strain, and colonies were assayed on three replicate plates. Positive controls were functional. The test article caused no increase in the reversion frequency. Some bacterial toxicity occurred as indicated by a drop in the reversion frequency for some tester strains at doses of approximately 500 ug/plate and higher. **No adverse effects indicated. Study supplemental** (Vidair 10/6/99).

52544-050; 169460; “Report on the Study of Reg. No. 292 932 in the Ames Salmonella/Mammalian Microsome Mutagenicity Test and Escherichia coli/Mammalian Microsome Reverse Mutation Assay, Test Substance: Plant Metabolite [BAS 490-9] of Kresoxim-methyl” (Hoffmann, H.D. and Engelhardt, G., BASF Aktiengesellschaft, Dept. of Toxicology, D-67056 Ludwigshafen/Rhein, Germany, Project ID No. 40M0263/944204, 12/30/94). *S. typhimurium* tester strains TA1535 and TA100 (reverted to histidine autotrophy by base substitution), strains TA1537 and TA98 (reverted by frameshift mutation), and *E. coli* tester strain WP2uvrA (reverted to tryptophan autotrophy by base substitution) were exposed to test article Reg. No. 292 932 (Batch No. 00436-69, 99.6% purity, plant metabolite BAS 490-9) at 0 (vehicle only), 4, 20, 100, 500, 2500 and 5000 ug/plate, with or without activation by an S-9 microsomal fraction. Exposure was at 37°C for 48 hours, with or without an additional 20 min 37°C pre-incubation period (in which the bacteria, S-9 fraction and test article were incubated together prior to the addition of agar). Each condition was tested once in each tester strain, and colonies were assayed on three replicate plates. Positive controls were functional. The test article caused no increase in the reversion frequency. Some bacterial toxicity occurred as indicated by a drop in the reversion frequency for some tester strains at doses of approximately 2500 ug/plate and higher. **No adverse effects indicated. Study supplemental** (Vidair 10/6/99).

52544-050; 169461; “Reverse Mutation Assay of Reg. No. 279 482 in Rats (Test Substance: Metabolite of Kresoxim-methyl)” (Nakajima, M., Biosafety Research Center, 582-2 Arahama, Shiohinden, Fukude-cho, Iwata-gun, Shizuoka, Japan, Project ID No. 3214(229-003), 2/12/97) *S. typhimurium* tester strains TA1535 and TA100 (reverted to histidine autotrophy by base substitution), strains TA1537 and TA98 (reverted by frameshift mutation), and *E. coli* tester strain WP2uvrA (reverted to tryptophan autotrophy by base substitution) were exposed to test article Reg. No. 279 482 (Lot No. 00689-83, 98.6% purity, metabolite of kresoxim-methyl) at 0 (vehicle only), 9.77, 19.5, 39.1, 78.1, 156, 313, 625, 1250, 2500 and 5000 ug/plate, with or without activation by an S-9 microsomal fraction. Exposure was at 37°C for 48 hours, with an additional 20 min 37°C pre-incubation period in which the bacteria, S-9 fraction (or buffer) and test article were incubated together prior to the addition of agar. Each condition was tested once in each tester strain (concentrations 9.77 through 78.1 for tester strain TA1537 only, in the absence of S-9 fraction), and colonies were assayed on two replicate plates. Positive controls were functional. The test article caused no increase in the reversion frequency. There was no bacterial toxicity, even though test article precipitation was noted at concentrations of 313 ug/plate and above. **No adverse effects indicated. Study supplemental** (Vidair 10/6/99).

CHROMOSOME EFFECTS

** 52544-022; 164391; “In Vitro Cytogenetic Investigation of Reg. No. 242 009 (BAS 490 F) in Human Lymphocytes” (Engelhardt, G., BASF Aktiengesellschaft, Dept. of Toxicology, D-67056 Ludwigshafen/Rhine, FRG, Project ID No. 30M0577/904521, 4/26/93). Human lymphocytes stimulated for 48 hours by PHA were exposed to 0, 10, 20 or 40 ug/ml of Reg. No. 242 009 (Batch No. N21; 98.7% purity) for 3 hours at 37°C, followed by washing and 21 hours of further incubation at 37°C, the last 2-3 hours in the presence of colcemid (duplicate cultures/dose). Metaphase cells were then collected and scored (100 cells/culture) for chromosome aberrations. Exposure of cells to the test article was in the presence or absence of an S9 microsomal fraction. Positive controls were functional. The test article did not induce either structural or numerical

(aneuploidy or polyploidy) aberrations. This was true for structural aberrations whether or not chromosome gaps were included in the analysis. There were also no effects on mitotic indices. The data show that the test compound did not cause chromosome damage in human lymphocytes under the conditions of this assay. **No adverse effects indicated. Study acceptable** (Vidair 9/15/99).

** 52544-022; 164392; "Cytogenetic Study In Vivo of Reg. No. 242 009 (BAS 490 F) in Mice: Micronucleus Test-Single IP Administration" (Engelhardt, G., BASF Aktiengesellschaft, Dept. of Toxicology, D-67056 Ludwigshafen/Rhine, FRG, Project ID No. 26M0180/914332, 10/7/93). Five male and five female mice/dose level were administered a single ip injection of Reg. No. 242 009 (Batch No. N36 (=IIIc1); 93.7% purity) at doses of 0 (vehicle only), 500, 1000 or 2000 mg/kg. After 16 hours (2000 mg/kg only), 24 hours (all dose levels and controls) or 48 hours (2000 mg/kg only) the animals were sacrificed and bone marrow was isolated from two femora per animal. Slides were prepared and scored for the frequencies and sizes of micronuclei per polychromatic or monochromatic erythrocyte. Positive controls were functional, both in terms of induction of micronuclei (cyclophosphamide and vincristine) and induction of large micronuclei (vincristine only). The test article did not inhibit erythropoiesis, since the ratio of polychromatic to normochromatic erythrocytes was unchanged. Furthermore, the numbers of both types of erythrocytes containing micronuclei were not increased by the test article. There was also no effect of the test compound on the average size of the micronuclei that were observed. Thus, under the conditions of this *in vivo* assay, Reg. No. 242 009 was not clastogenic and did not impair chromosome segregation. **No adverse effects indicated. Study acceptable** (Vidair 9/15/99).

** 52544-050; 169462; "Cytogenetic Study In Vivo with Reg. No. 242 009 in the Rat Micronucleus Test; Single Intraperitoneal Administration" (Engelhardt, G., Hoffmann, H.D., BASF Aktiengesellschaft, Dept. of Toxicology, D-67056 Ludwigshafen/Rhein, Germany, Project ID No. 06M0180/914384, 4/1/97). Five male and five female rats/dose level/time point were administered a single intraperitoneal injection of Reg. No. 242 009 (Batch No. N36=IIIc1; 94.9% purity) at doses of 0 (0.5% carboxymethyl cellulose), 500, 1000 or 2000 mg/kg. After 24 h (all dose levels, positive and negative controls) or 48 h (2000 mg/kg and negative control) the animals were sacrificed and bone marrow isolated from one femur per animal. Bone marrow smears were prepared and scored for frequencies and sizes of micronuclei per polychromatic or normochromatic erythrocyte. By 24 h after injection, the positive control (20 mg/kg of cyclophosphamide) induced an approximate 5-fold higher frequency of polychromatic erythrocytes with micronuclei compared to the vehicle-only control. The frequency of normochromatic erythrocytes with micronuclei was unaffected. The test article caused no significant increase in the frequency of either cell type with large or small micronuclei, either at 24 or 48 h post-injection. It was concluded that the test article was neither clastogenic nor a spindle poison, since chromosomes segregated normally at all dose levels. **No adverse effects indicated. Study acceptable** (Vidair 10/8/99).

DNA DAMAGE

** 52544-022; 164394; "In Vitro Unscheduled DNA Synthesis (UDS) Assay in Rat Hepatocytes with Reg. No. 242 009 (BAS 490 F)" (Polloth, C., BASF Aktiengesellschaft, Dept. of Toxicology, D-67056 Ludwigshafen/Rhine, FRG, Project ID No. 81M0180/914334, 5/13/94). Primary cultures of rat hepatocytes were incubated for 18 hours with 37 MBq/ml of ³H-Thymidine and the following concentrations of Reg. No. 242 009 (Batch No. N36 (III c 1); 94.3% purity): 0

(2% DMSO), 0.33, 1.0, 3.3, 33 and 100 ug/ml. Cells were processed for autoradiography and 100 cells/dose level were examined for silver grains in the nucleus and cytoplasm. Two identical experiments were performed. At ≥ 33.3 ug/ml of test article, cytotoxicity precluded performance of the assay. Up to 10 ug/ml there was a maximum of 2% of cells undergoing unscheduled DNA synthesis, measured as cells with net nuclear grain counts of ≥ 5 . In contrast, the positive control (10 uM 2-acetylaminofluorene) induced 41% and 52% of cells to undergo unscheduled DNA synthesis for the two experiments. Therefore, the test article was considered inactive in the assay. **No adverse effects indicated. Study acceptable** (Vidair 9/17/99).

** 52544-022; 164395; “Ex Vivo Unscheduled DNA Synthesis (UDS) Assay in Rat Hepatocytes with Reg. No. 242 009 in Rats After Administration in the Diet for 3 Weeks” (Polloth, C., BASF Aktiengesellschaft, Dept. of Toxicology, D-67056 Ludwigshafen/Rhine, FRG, Project ID No. 80M0180/914336, 10/5/94). Reg. No. 242 009 (Batch No. N36 (IIIc1); 94.3% purity) was administered to 3 male rats/dose level in their feed for 3 weeks at 200 and 16000 ppm. Positive (2-acetylaminofluorene, 50 mg/kg) and negative controls (0.5% carboxymethylcellulose, 10 ml/kg) were administered by single-dose gavage, followed by an 18 hour interval. Hepatocytes from treated and control animals were seeded onto glass coverslips and labeled with medium containing ^3H -thymidine for 4 hours at 37°C . Following autoradiography, 100 cells/animal were examined for silver grains over the nucleus and cytoplasm. The test compound did not cause any cytotoxicity. There were no test compound-induced increases in the mean net grain counts per nucleus, and the mean percentages of cells with net nuclear grain counts of ≥ 5 was 0 for all treatment levels. In contrast, the positive control yielded 47% of the cells with net nuclear grain counts of ≥ 5 , indicating unscheduled DNA synthesis in those cells. Thus, the test compound was inactive in this assay of DNA damage and its repair. **No adverse effects indicated. Study acceptable** (Vidair 9/17/99).

** 52544-022; 164396; “Ex Vivo Unscheduled DNA Synthesis (UDS) Assay and S-Phase-Response in Rat Hepatocytes with Reg. No. 242 009 in Rats” (Polloth, C., BASF Aktiengesellschaft, Dept. of Toxicology, D-67056 Ludwigshafen/Rhine, FRG, Project ID No. 82M0180/914337, 9/26/94). Reg. No. 242 009 (Batch No. N36(IIIc1); 94.3% purity) was administered to 3 male rats/dose level by oral gavage at 20, 200 and 1000 mg/kg. After 18 hours the animals’ livers were removed and hepatocytes isolated. Labeling hepatocytes with ^3H -thymidine for 4 hours at 37°C allowed quantification of the fractions of cells in S phase and the fractions undergoing unscheduled DNA synthesis. The test compound caused no cytotoxicity. There were no significant compound-induced increases in mean net grain counts per cell, and the mean percentages of cells with net grain counts of ≥ 5 was less than 1% for cultures derived from treated animals (compared to 47% for the positive control). These results suggest that Reg. No. 242 009 does not induce DNA damage and repair. The fractions of hepatocytes in S phase were elevated for cultures from treated animals relative to the negative control: 1.0, 1.4, 2.8 and 2.6 for 0, 20, 200 and 1000 mg/kg, respectively. The positive control (4-acetylaminofluorene) had 5.9% of the cells in S phase. The data suggest that the test article did stimulate the proliferation of hepatocytes. However, the large variability between animals in the same treatment groups (4 to 14.5-fold variation in S phase fractions) casts doubt on the reliability of this conclusion. **No adverse effects indicated. Study acceptable** (Vidair 9/21/99).

NEUROTOXICITY

** 52544-044; 169445; “BAS 490 R (Reg. No. 242 009): Acute Oral Neurotoxicity Study in Wistar Rats” (Mellert, W., Kaufmann, W. and Hildebrand, B., BASF Aktiengesellschaft, Dept. of Toxicology, D-67056 Ludwigshafen/Rhine, FRG, Project ID No. 20C0180/91104, 6/27/96). Ten rats/sex/dose level were administered Reg. No. 242 009 (Batch No. N36=IIIc1; 93.7% purity) by single-dose oral gavage at 0 (0.5% carboxymethyl cellulose), 500, 1000 and 2000 mg/kg. Functional observational batteries and measurements of motor activity were performed at -6, 0, 7 and 14 days (0=day of dosing). Neuropathological examinations were performed at day 14. In addition, bodyweights were measured weekly and the animals checked for clinical signs daily. No treatment-related effects were observed on any of the indices of neurological function. Bodyweights, clinical signs, and neuropathological examinations were all normal. **No adverse effects indicated. NOEL (M/F) = 2000 mg/kg** (based on the absence of test compound-induced effects in animals dosed with 2000 mg/kg). **Study acceptable** (Vidair 10/4/99).

(See also 90 day subchronic neurotoxicity study in following section)

SUBCHRONIC STUDIES

52544-009; 164356; “Subchronic Toxicity with Reg. No. 242 009 (BAS 490 F) in Mice: Administration in the Diet Over 3 Months” (Mellert, W., BASF Aktiengesellschaft, Dept. of Toxicology, D-67056 Ludwigshafen/Rhine, FRG, Project ID No. 53S0577/90065, 6/27/94). Test article Reg. No. 242 009 (Batch No. N21; 98.7% purity) was mixed with feed and offered ad libitum to 10 male and 10 female mice/dose level for 90 days at the following concentrations: 0, 250, 1000, 4000 or 8000 ppm. The mean daily intake of test article in ascending order of feed concentration was: males 0/57/230/909/1937 mg per kg; females 0/80/326/1326/2583 mg per kg. There was no mortality and the few clinical signs did not indicate a toxicological effect of the test article. Bodyweights of treated animals increased similarly to those of controls. Hematology and serum chemistry revealed no compound-related alterations of statistical significance. At necropsy, absolute liver weights of animals fed 4000 or 8000 ppm were higher than those of controls (3% and 7%, respectively), though not significantly so. Upon normalization of liver weights to the bodyweights of the necropsied animals, males fed 4000 or 8000 ppm had relative liver weights which were significantly higher than those of controls (11% or 19%, respectively). Histopathology could not identify any compound-related effects on the liver responsible for the increase in relative liver weight (dilation of the uterus in 3 females fed the highest dose was a possible compound-related effect). Gross pathology also failed to detect any consistent abnormalities in any organ or tissue. **No adverse effects indicated. NOEL (M/F) = 8000 ppm** (based on the probable absence of compound-induced toxicological effects at 8000 ppm). **Study not acceptable** due to lack of ophthalmology (Vidair 8/12/99).

** 52544-010; 164357; “Subchronic toxicity with Reg. No. 242 009 (BAS 490 F) in Rats: Administration in the Diet Over 3 months” (Mellert, W., BASF Aktiengesellschaft, Dept. of Toxicology, D-67056 Ludwigshafen/Rhine, FRG, Project ID No. 31S0577/90041, 10/24/94). Test article Reg. No. 242 009 (Batch No. N21, 98.7% purity) was mixed with feed and offered ad libitum to 10 male and 10 female rats/dose level for 90 days at the following concentrations: 0, 500, 2000, 8000, 16000 ppm. The mean daily intake of test article in ascending order of

concentration in the feed was: males 0/36/146/577/1170 mg/kg; females 0/43/172/672/1374 mg/kg. There was no mortality, no abnormal clinical signs, and food consumption was not significantly affected. Bodyweights of males were significantly lower than controls in the two highest dose groups. At 8000 ppm they were significantly lower (7-10%) for 10/14 weeks while at 16000 ppm they were significantly lower (7-8%) for only 3/14 weeks. Females fed the two highest doses had bodyweights that trended lower than controls, but the differences were never significant. Hematology and blood chemistry were normal, except for some variations in enzyme levels. Aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase decreased significantly in treated males and females (only the latter two in females). However, these changes were usually not consistent over the two time points tested (days 43 and 89), and showed no clear dose-response. In contrast, glutamyl transferase increased in males fed 8000 and 16000 ppm to 4.7 fold and 5.5 fold of control values, respectively. At necropsy there were no significant changes in absolute organ weights. However, both treated males and females exhibited significant increases in relative liver weights: males at 16000 ppm (110% of controls) and females at 2000 ppm (110%), 8000 ppm (107%), and 16000 ppm (112%). The kidneys and adrenal glands of treated males also showed significant increases over controls: kidneys 108-113% of control values for 2000, 8000, and 16000 ppm; adrenal glands 124% of controls for 8000 ppm. Gross pathological exams did not identify any treatment-related changes in the above organs, or in any other organs or tissue. Two changes at the microscopic level were evident: increased kidney calcification in treated males (number of animals affected: 0/0/0/2/1 for 0/500/2000/8000/16000 ppm) and reduced diffuse fatty infiltration of the liver in treated females (number of animals affected: 9/9/6/3/2 for 0/500/2000/8000/16000 ppm). The toxicological significance of these microscopic changes is not apparent at this time. **No adverse effects indicated. NOEL (M) = 2000 ppm** (146 mg/kg; based on reduced bodyweights, increased serum glutamyl transferase, increased relative kidney weights, and increased kidney calcification in animals fed 8000 ppm); **(F) = 500 ppm** (43 mg/kg; based on increased relative liver weights and reduced diffuse fatty infiltration of the liver in animals fed 2000 ppm). **Study Acceptable** (Vidair 8/13/99).

52544-011; 164358; "S-Phase Response with Reg. No. 242 009 in Rats After Administration in the Diet for 3 Weeks" (Polloth, C., BASF Aktiengesellschaft, Dept. of Toxicology, D-67056 Ludwigshafen/Rhine, FRG, Project ID No. 83M0180/910149, 10/5/94). Five male rats/dose were offered feed containing 0, 200 or 16000 ppm of test article Reg. No. 242 009 (Charge No. N36(IIIc1); purity = 94.3%) for 3 weeks. Mean daily test compound intake was 15 and 1140 mg/kg for 200 and 16000 ppm, respectively. During the last week, osmotic minipumps were implanted so as to provide continuous perfusion by BrdU and allow identification of proliferating cells. Test article administration for 3 weeks had no effect on mortality, bodyweights or food consumption, and no abnormal clinical signs were seen. At necropsy, absolute and relative liver weights were not affected by the test compound. Gross pathology and histopathology identified no test article-related abnormalities in any organs or tissue. However, labeling indices of hepatocytes in zone 1 of the hepatic lobule were significantly higher (about 3 fold) for the 16000 ppm dose group compared to controls. A smaller 2 fold increase was observed when zones 1, 2 and 3 were measured, perhaps reflecting the fact that zone 1 cells have more ready access to fresh blood entering the liver. The stimulation of proliferation was only induced by the high dose of test article, and was greater in the right medial lobe (3.7 fold) and right lateral lobe (3.6 fold) than in the processus caudatus (2.5 fold). **NOEL (M) = 200 ppm** (based upon an increased labeling index for hepatocytes from male rats fed 16000 ppm for 3 weeks). **Supplemental Study** (Vidair 8/17/99).

52544-011; 164359; "S-Phase Response Study with BASF 490..F(Reg. No. 242 009) in 16-Month Old Wistar Rats After Administration in the Diet for 3 Weeks" (Polloth, C., BASF Aktiengesellschaft, Dept. of Toxicology, D-67056 Ludwigshafen/Rhine, FRG, Project ID No. 83M0180/91162, 11/15/94). Fifteen month old male rats were offered test article Reg. No. 242 009 (Charge No. N36(IIIc1); purity = 94.3%) in their feed for 3 weeks, at concentrations of 0, 200 or 16000 ppm, 5 animals per dose level. During the third week, osmotic minipumps were implanted so as to provide continuous perfusion of BrdU and allow identification of proliferating cells. Results were similar to those of an earlier study with 9 week old rats (Record #164358). There were no effects on mortality or bodyweight, and no clinical signs. At necropsy, absolute and relative liver weights were unaffected, and gross/histopathology were normal. However, 16000 ppm of test article induced an approximate 3 fold increase in the zone 1 labeling index of hepatocytes which was statistically significant. At 200 ppm there was no significant effect. The increase in labeling index at 16000 ppm was somewhat smaller in the processus caudatus (2.7 fold) compared to the medial (3.3 fold) and lateral (2.9 fold) lobes. These results show that age is not a factor in the test article-induced stimulation of liver cell proliferation. Furthermore, since liver weight and pathology (both gross and microscopic) were unaffected at 16000 ppm, the toxicological significance of the stimulation of proliferation must be questioned. **NOEL (M) = 200 ppm** (based upon an increased labeling index for hepatocytes from fifteen month old rats fed 16000 ppm for 3 weeks). **Supplemental Study** (Vidair 8/17/99).

52544-011; 164360; "S-Phase Response Study with BASF 490..F (Reg. No. 242 009) in Wistar Rats After Administration in the Diet for 3 Weeks" (Mellert, W., BASF Aktiengesellschaft, Dept. of Toxicology, D-67056 Ludwigshafen/Rhine, FRG, Project ID No. 99C0180/91182, 3/20/97). Five male rats per dose group were offered 0, 800 or 8000 ppm of Reg. No. 242 009 (Charge No. N36(IIIc1); purity = 94.9%) in their feed for 3 weeks, the last week with an implanted osmotic minipump to provide perfusion by BrdU and allow identification of proliferating cells. Mean daily intake of test article was 61 and 603 mg/kg for 800 and 8000 ppm, respectively. There were no compound-related effects on mortality, bodyweight, absolute or relative liver weight, or gross/histopathology. There was a significant 6% decrease in food consumption in the 8000 ppm dose group for week 1, but not for weeks 2 or 3. 8000 ppm of test article also caused a significant increase in the hepatocyte labeling index, measured in both zone 1 cells (almost 2 fold increase) and zone 1 + zone 2 cells (36% increase). The stimulation was similar (69-94%) for the 3 lobes (processus caudatus, medial, lateral). 800 ppm did not significantly affect the labeling indices. **NOEL (M) = 800 ppm** (based upon an increased labeling index for hepatocytes from male rats fed 8000 ppm for 3 weeks). **Supplemental Study** (Vidair 8/18/99).

** 52544-012; 164363; "Study on the Dermal Toxicity of Reg. No. 242 009 (BAS 490 F) in Rats: Application to the Intact Skin Over 3 Weeks (21 Applications)" (Kirsch, P., BASF Aktiengesellschaft, Dept. of Toxicology, D-67056 Ludwigshafen/Rhine, FRG, Project ID No. 37H0180/91094, 11/23/94). Test article Reg. No. 242 009 (Batch No. N36=IIIC1; purity = 94.3%) was applied to clipped dorsal skin sites on rats for at least 6 hours per day for 21 consecutive days. Five animals/sex/dose group received either 0 or 1000 mg/kg. At study's end, the animals were bled, sacrificed and subjected to necropsy. There were no compound-related effects on food consumption, bodyweights, mortality, clinical signs or skin test sites. Hematology and blood chemistry were also normal. Gross pathological examinations revealed no changes to any organs or tissue. Lastly, the histopathology of the treated skin, normal skin, liver and kidneys was unaffected by the test compound. **Systemic and Dermal NOEL (M/F) = 1000 mg/kg** (based upon

the absence of toxicological effects or skin irritation in animals receiving applications of the test compound at 1000 mg/kg for 21 consecutive days). **Study Acceptable** (Vidair 8/19/99).

** 52544-046; 169448; "BAS 490 F (Reg. No. 242 009): Subchronic Oral Neurotoxicity Study in Wistar Rats" (Mellert, W., Kaufmann, W. and Hildebrand, B., BASF Aktiengesellschaft, Dept. of Toxicology, D-67-56 Ludwigshafen/Rhine, FRG, Project ID No. 50C0180/91164, 6/27/96). Ten rats/sex/dose level were administered Reg. No. 242 009 (Batch No. N36(IIIc1); 94.3% purity) in their feed for 3 months at 0, 1000, 4000 and 16000 ppm. Observations of the animals' general state of health included clinical signs, food consumption and bodyweight. Functional observational batteries and measurements of motor activity were performed on days -7, 22, 50 and 85 (0=day of dosing). Neuropathological examinations, including brain weights, were performed at study's end. Mean test substance intake over the 3 month study interval for 1000/4000/16000 ppm was: males 72/292/1180 mg/kg/day; females 84/341/1354 mg/kg/day. There was no evidence of neurotoxicity and neuropathology was normal. Only sporadic effects on food consumption and bodyweights were noted in the highest dose group (male mean bodyweights 8% lower than controls by day 91, difference not significant; females 9% lower by day 91, $p < .01$). **No adverse effects indicated. Non-neurological NOEL (M/F) = 4000 ppm** (based on sporadically lower mean bodyweights and food consumption in animals fed 16000 ppm); **Neurological NOEL (M/F) = 16000 ppm** (based on the absence of neurotoxicity in animals fed 16000 ppm). **Study acceptable** (Vidair 10/4/99).

METABOLISM STUDIES

** 52544-023; 164398; "The Metabolism of ^{14}C -Reg. No. 242 009 (BAS 490 F) in Rats" (Kohl, W., BASF Aktiengesellschaft, Ecology and Environmental Analytics, Metabolism, Postfach 120, D-67114 Limburgerhof, FRG, Laboratory Study Code P90-M009, 11/17/94). Five male and five female rats were administered ^{14}C -labeled Reg. No. 242 009 (ring B-labeled: Lots 308-5, 353-01, 422-03, 445-25, radiochemical purity $\geq 99\%$, chemical purity $\geq 99\%$; ring A-labeled: Lot 358-01, radiochemical purity $\geq 98\%$, chemical purity $> 98\%$) according to each of 4 protocols: 1) single i.v. dose at 5 mg/kg, 2) single oral dose at 50 mg/kg, 3) fourteen daily oral doses of unlabeled test compound at 50 mg/kg followed by a single dose of labeled compound at 50 mg/kg, 4) single oral dose at 500 mg/kg. Additional animals were dosed as needed to provide more material for analysis of metabolites. Metabolite identifications and structural determinations were made by HPLC, mass spectrometry and NMR spectroscopy. From 66 to 81% of the oral dose was excreted in the feces, with up to 75% of the administered dose being in the form of the unchanged parent compound, indicating that the test compound was poorly absorbed from the g.i. tract. Following i.v. injection, male excretion of the parent compound was not detectable and female excretion was only 8% and 16% of the administered dose for the feces and urine, respectively, indicating that the test compound was readily metabolized to other products. This was confirmed in orally dosed animals, where only metabolites of the parent compound were detected in the bile, plasma, liver and kidneys. Thirty-four metabolites were identified. Phase I biotransformation reactions included: 1) cleavage of ester bonds, 2) cleavage of oxime ether bonds, 3) cleavage of benzyl ether bonds, 4) hydroxylation of the A ring, and 5) oxidation of the aryl-methyl group to benzyl alcohol followed by oxidation to carboxylic acid. Most of the hydroxyl groups which were produced underwent Phase II conjugation with glucuronic acid or sulphate, resulting in a large number of new metabolites. The most important first-pass biotransformation reaction was ester cleavage of the parent compound leading to formation of the free acid M1. M1 was most

commonly metabolized via hydroxylation on the A ring at the aryl-CH₃ group to produce M2 and M9, which along with their glucuronides were the main products in the excreta. Males, and to a lesser extent females, were able to cleave the benzyl ether bridge, thereby separating the A and B rings. The B ring-containing intermediate was demethylated to form M6, which was transformed into its cyclisation product M20. The A ring-containing intermediate was further oxidized to monohydroxybenzyl alcohol, dihydroxybenzyl alcohol, salicylic acid and o-cresol, all found only in conjugated forms. Administration of 14 consecutive daily doses of test compound failed to change the metabolite pattern of the fifteenth dose, indicating that biotransformation enzyme systems were not induced by the test article and bioaccumulation did not occur. **Study acceptable** (Vidair 9/23/99).

52544-024; 164399; “¹⁴C-Reg. No. 242 009 (BAS 490 F): Quantitative Whole-Body Autoradiography Following Oral Administration to the Rat” (Whitby, B.R., Hazleton UK (HUK), Otley Road, Harrogate, North Yorkshire, England, HUK Project No. 729/166, 7/20/93). Five male and five female rats were administered ¹⁴C-Reg. No. 242 009 (Batch No. 353-01, 30.2 mCi/mmol, 99.2% radiochemical purity) at 50 mg/kg by oral gavage. At 0.5, 2, 8, 24 and 96 h post-dosing, an animal of each sex was sacrificed and processed for whole body autoradiography to determine the tissue distribution of the test article. Radioactivity in sagittal sections was quantified by image analysis. Most radioactivity was confined to the g.i. tract over the course of the experiment, and could not be quantified due to film overexposure. Of the labeled test article which was absorbed, the highest levels were measured in the liver (3.854-5.269 ug equiv./g tissue) and kidneys (5.157-6.514 ug equiv./g tissue) at 0.5 and 2 hours post-dosing. These amounts declined to less than 1 ug equiv./g tissue by 24 hours. Only one other tissue had more than 1 ug equiv./g tissue of test article: 1.3 ug equiv./g tissue in the uveal tract of the male sacrificed at 2 h, declining to non-quantifiable levels thereafter. These data suggest that the test article does not accumulate in any of the tissues examined. **Supplemental study** (Vidair 9/28/99).

** 52544-024; 164400; “Study of the Biokinetics of ¹⁴C-Reg. No. 242 009 (BAS 490 F) in Rats” (Gans, G., BASF Aktiengesellschaft, Dept. of Toxicology, D-67056 Ludwigshafen/Rhine, FRG, Project ID No. 02B0547/906004, 11/10/94). A total of 60 male and 60 female rats were administered ¹⁴C-labeled Reg. No. 242 009 (ring B-labeled: Lots 308-5, 353-01, 422-03, 445-25, radiochemical purity > 98%, chemical purity > 97%; ring A-labeled: Lot 358-01, radiochemical and chemical purity > 98%) according to one of 4 protocols: 1) single i.v. dose of 5 mg/kg, 2) single oral dose of 50 mg/kg, 3) single oral dose of 500 mg/kg, 4) fourteen daily oral doses of unlabeled compound at 50 mg/kg followed by a single dose of labeled compound at 50 mg/kg. Excretion of the labeled compound was measured in the urine and feces for up to 120 h post-dosing. Plasma and tissue levels of the test article were also monitored for up to 120 h. Bile duct-cannulated animals were used to measure the concentration in the bile. After oral dosing, generally over 90% of the administered dose was excreted by 120 h. No radioactivity was detected in the air exhaled by animals dosed orally and monitored for 48 h. Most was excreted in the feces (81% for 500 mg/kg, ring B; 66-67% for 50 mg/kg, ring B; 62-78% for 500 mg/kg, ring A). Increasing the dose from 50 mg/kg to 500 mg/kg caused an approximate halving of the percentage of the administered dose that was excreted in the urine, suggesting that absorption and/or metabolism of the ring B-labeled compound was saturated at the high dose. Intravenous administration resulted in more than twice as much excretion via the urine compared to oral dosing, confirming that the test compound was only slowly absorbed after oral dosing. After oral dosing, plasma T_{max} levels were reached by 0.5-1 h (50 mg/kg) and 8 h (500 mg/kg), with terminal half lives of 16.9-19.1 h (50 mg/kg) and 22.1-30.5 h (500 mg/kg). When the plasma concentration

was plotted versus time, the area under the curve (AUC) values for 500 mg/kg were only 2.1-2.3 fold higher than for 50 mg/kg, again indicating that absorption/excretion was saturated at the higher dose. Biliary excretion by 48 h after oral dosing was 35-43% of the administered dose for 50 mg/kg and 14-15% for 500 mg/kg. The total amount of test article excreted in the bile + urine, reflective of the compound's bioavailability, was approximately 63% for 50 mg/kg and 23-27% for 500 mg/kg, again indicating saturation of absorption and/or metabolism at the high dose level. Tissue levels were highest in the g.i. tract, plasma, liver, kidneys and adrenals and lowest in muscle and brain. By 96 h following oral dosing, all tissues contained less than 1 ug equiv./g of the test article. Repeated daily dosing had no effect on the pattern of test article excretion or retention. **Study acceptable** (Vidair 9/27/99).

52544-051; 169463; "In Vitro Comparison of the Metabolism of ¹⁴C-BAS 490 F (14C-242 009) in Rats and Mice" (Salmon, F., BASF Aktiengesellschaft, Animal Metabolism and Residue, Postfach 120, D-67114 Limburgerhof, FRG, Project ID No. 33079, July 1996). Plasma and hepatocytes isolated from rats and mice, in combination with HPLC analysis, were used to identify potential species-specific differences in the metabolism of ¹⁴C-BAS 490 F (Batch No. 445-25, radiochemical purity 99.2%, chemical purity 97.2%). In rats, the primary metabolic effect on the test article was cleavage of the ester bond to generate the free acid 490 M1 (established by Study # 164398). The kinetics of 490 M1 formation, following addition of the radiolabeled test article to plasma, were similar for both species and both sexes: formation of 490 M1 was almost complete after 15 min at 37°C, and the half-life of the parent compound fell somewhere between 2 and 5 minutes. Metabolite patterns from isolated hepatocytes were similar for the two species, with only minor quantitative differences: male rats produced more H5 and H6 metabolites (hydroxylated derivatives of 490 M1) than female rats or mice, and mice generated an unknown peak that was not seen in rats (probably metabolite 490 M24, identified in rat urine and feces in Study #164398, but present at undetectable levels in the instant study). It was concluded that rats and mice have only minor quantitative differences in the way they metabolize the test article. **Study supplemental** (Vidair 10/8/99).

52544-051; 169464; "BAS 490 F (Reg. No. 242 009): Study of Enzyme Excretion in Urine of Wistar Rats After Repeated Administration in the Diet" (Mellert, W., Deckardt, K. and Hildebrand, B., BASF Aktiengesellschaft, Dept. of Toxicology, D-67056 Ludwigshafen/Rhein, Germany, Project ID No. 99c0180/91183, 3/20/97). Ten rats/sex/dose level were administered BAS 490 F (Batch No. N36(IIIc1); 94.9% purity) in their feed for 2 weeks at 0 and 16000 ppm. As had been observed in previous feeding studies with rats, both sexes fed 16000 ppm of test article for 2 weeks exhibited approximate 25% (p<.001) decreases in serum alanine aminotransferase and alkaline phosphatase activity relative to controls. To determine if the test article induced enzyme loss through the urine, urinalysis was also performed at 2 weeks. No significant effects were measured in either the concentrations of enzymes excreted in the urine or in the amounts of enzymes excreted over time. Consistent with this, the rate of creatinine excretion and the urine volume were also normal. Therefore, the test article-induced decreases in serum enzyme levels were not caused by increased rates of their excretion in the urine. The data suggest that the test article does not cause kidney toxicity, and indicate that the drops in serum enzyme levels have a different etiology. **Study supplemental** (Vidair 10/8/99).