[OPP-35000/2; PH-FRL 1700-6]

Determination Not To Initiate a Rebuttable Presumption Against Registration (RPAR) of Pesticide Products Containing Carbaryl; Availability of Decision Document

AGENCY: Environmental Protection Agency (EPA).

ACTION: Notice of Determination not to initiated an RPAR.

SUMMARY: The EPA has concluded not to issue a rebuttable presumption against registration for carbaryl, a broad-spectrum pesticide currently registered by EPA for use as an insecticide/acaricide, and as a plant regulator. The Agency has determined to return carbaryl to the registration process; however, the Agency will require from registrants additional data to support existing registrations pursuant to section 3(c)(2)(B) of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), as amended, and will negotiate appropriate label changes, outside the RPAR process, to ensure that exposure to carbaryl is held to reasonable levels.

ADDRESS: Copies of the Decision Document of Carbaryl are available from: Juanita Wills, Special Pesticide Review Division (TS-791), Office of Pesticide Programs, Room 711, Crystal Mall No. 2, 1921 Jefferson Davis Highway, Arlington, Virginia 22202.

FOR FURTHER INFORMATION CONTACT: Juanita Wills (703-557-7420).

SUPPLEMENTARY INFORMATION:

I. Introduction

Carbaryl is a broad-spectrum insecticide/acaricide as well as a plant regulator used in thinning apples. Its major uses include applications to cotton, peanuts, soybeans, field and sweet corn, ornamentals and turf, forest and shade trees, deciduous tree fruits, many other fruit, vegetable, and nut crops, poultry, and pets. EPA has reviewed the risks associated with the use of carbaryl to determine whether a Rebuttable Presumption Against Registration (RPAR) should be issued for carbaryl. The Agency submitted carbaryl to the Special Pesticide Review Division (SPRD) as a candidate for the RPAR process primarily because a laboratory study conducted in 1968 found carbaryl to be teratogenic when administered in low doses to pregnant beagle dogs. A 1969 study of beagles also showed positive results. In addition to teratogenicity and fetotoxicity, the Agency was concerned that use of carbaryl had the potential to cause the following effects: mutagenicity, oncogenicity, neurotoxicity, and viral enhancement. This Notice sets forth the Agency's determinations with regard to the potential of carbaryl to produce these adverse effects of concern. The Agency has concluded that the overall weight of evidence of the extensive data which are currently available does not indicate that risk criteria warranting a Rebuttable Presumption Against Registration for carbaryl have been met or exceeded. Accordingly, the Agency has determined to return carbaryl to the registration process. Independent of the Agency's determination not to issue a Rebuttable Presumption Against Registration of carbaryl pesticide products, the Agency has determined that the following measures are warranted and will be pursued: (1) requirements for additional data to support the existing registrations, pursuant to section 3(c)(2)(B) of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), as amended; (2) appropriate label changes to be implemented according to negotiations between the Agency and registrants to ensure that exposure to carbaryl is held to reasonable levels.

This Notice is organized into four units. Unit I is this Introduction. Unit II, entiled "Legal Background", sets forth a general discussion of the regulatory

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framework within which these actions are taken. Unit III, and the accompanying Decision Document, set forth the determinations which the Agency has reached and the bases for these determinations. Unit IV, entitled "Procedural Matters", provides a brief discussion of the procedures which will be followed in implementing the determinations announced in this Notice.

II. The RPAR Process—Legal Background

In order to obtain a registration for a pesticide under FIFRA, a manufacturer must demonstrate that the pesticide statisfies the statutory standard for registration. That standard requires, among other things, that the pesticide perform its intended function without causing "unreasonable adverse effects on the environment" (Sec. 3(c)(5)). The term "unreasonable adverse effect on the environment" is defined as "any unreasonable risk to man or the environment, taking into account the economic, social and environmental costs and benefits of the use of any pesticide" (Sec. 2(bb)). In effect, this standard requires a finding that the benefits of each use of the pesticide exceed the risks of use, when the pesticide is used in accordance with commonly recognized practices. The burden of proving that a pesticide satisfies the registration standard is on the proponent of registration and continues as long as the registration remains in effect. Under Sec. 6 of FIFRA, the Administrator is required to cancel the registration of a pesticide or modify the terms and conditions of registration whenever he determines that the pesticide no longer satisfies the statutory standard for registration.

The Agency created the RPRA process to facilitate the identification of pesticide uses which may not satisfy the statutory standard for registration and to provide a public, informal procedure for the gathering and evaluation of information about the risks and benefits of these uses. The regulations governing the RPAR process are set forth at 40 CFR 162.11. In broad summary, these regulations set forth certain criteria of risk and provide that an RPAR shall arise against a pesticide if the Agency determines that the ingredient(s), metabolite(s), or degradation product(s) of the pesticide in question meet or exceed any of these risk criteria.

In administering the RPAR process, the Agency adheres to the standard for initiating the RPAR process established by Sec. 3(c)(8), one of the 1978 Amendments to FIFRA, which provides that the Agency may not start and RPAR unless it has "a validated test or other significant evidence raising prudent concerns of unreasonable adverse risk to man or to the environment." In determining whether a particular pesticide raises "prudent concerns," the Agency examines the degree of toxicity of the pesticide as indicated in laboratory studies and attempts to extrapolate the degree of risk which is likely to be posed to humans and the environment. In making this kind of extrapolation, the Agency evaluates the quality and adequacy of all available data before proceeding with an RPAR. This approach allows the Agency to avoid the burdensome consequences of an RPAR proceeding in those case where studies of questionable validity have called the safety of a pesticide into question but where the overall toxicological profile does not indicate that prudent concerns are warranted.

The Agency generally announces that an RPAR has arisen by publishing a notice in **Federal Register**. After an RPAR is issued, registrants and other interested persons are invited to review the data upon which the presumption is based and to submit data and information to rebut the presumption. Respondents may rebut the presumption of risk by showing that the Agency's initial determination of risk was in error, or by showing that use of the pesticide is not likely to result in any significant exposure to humans or to animals or plants of concern with regard to the adverse effects in question. See 40 CFR 162.11(a)(4). Further, in addition to submitting evidence to rebut the risk presumption, respondents may submit evidence as to whether the economic, social, and environmental benefits of the use of the pesticide subject to the presumption outweigh the risks of use.

The regulations require the Agency to conclude an RPAR by issuing a Notice of Determination in which the Agency states and explains its position on the question of whether the risk presumptions have been rebutted. If the Agency determines that a presumption is not rebutted, it will then consider information relating to the social, economic and environmental costs and benefits which registrants and other interested persons submitted to the Agency, and any other benefits information known to the Agency.

After weighing the risks and the benefits of a pesticide's uses, the Administrator may conclude the RPAR process by issuing a notice of intent to cancel or deny registration pursuant to FIFRA Sec. 6(b)(1) and Sec. 3(c)(6) of by issuing a notice of intent to hold a hearing pursuant to Sec. 6(b)(2) of FIFRA to determine whether the registration should be cancelled or applications for registration denied.

In determining whether the use of a pesticide poses risks which are greater than benefits, the Agency considers modifications to the terms and conditions of registration which can reduce risks, and the impact of such modifications on the benefits of the use. Among the risk reduction measures short of cancellation which are available to the Agency are changes in the directions of use on the pesticide's labeling and classification of the pesticide for "restricted use" pursuant to FIFRA Sec. 3(d).

III. Determinations and Regulatory Conclusions

A. Determinations on Risk

The Agency has considered information on the potential of carbaryl to produce teratogenicity and fetotoxicity, mutagenicity, neurotoxicity, and viral enhancement. The Agency's conclusions regarding these potential effects as based on currently available data are summarized below.

1. *Teratogenicity and Fetotoxicity.* 40 CFR 162.11(a)(3)(ii)(B) provides that a rebuttable presumption against a pesticide's registration and continued registration shall arise if that pesticide's ingredient(s) "produces any * * * chronic or delayed toxic effect [other than an oncogenic or mutagenic effect] in test animals at any dosage up to a level, as determined by the Administrator, which is substantially higher than that to which humans can reasonably be anticipated to be exposed, taking into account an ample margin of safety."

Data concerning the potential of carbaryl to induce adverse prenatal effects in mammalian species are extensive, more extensive than has been the case for other pesticides which have come under Agency review. As discussed in the introduction of this Notice, carbaryl became a candidate for the RPAR process because a laboratory study (Smalley, 1968) found carbaryl to be tertatogenic when administered to low doses to pregnant beagle dogs. A subsequent study (Imming et al., 1969) in beagle dogs also reported positive results. In addition, numerous studies have been conducted on diverse other mammalian species including the mouse, rat, gerbil, hamster, guinea pig, rabbit, swine, sheep, and monkey. All of the studies are not of equal utility, however, for purposes of assessing the potential of carbaryl to act as a perinatal toxicant in the environment. A number of the studies were done with inappropriate

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protocols, and some of the older American studies, as well as many of the foreign papers, are seriously flawed by inadequate presentation of data. The Agency has therefore chosen to base its conclusions concerning the potential teratogenic and fetotoxic risk from carbaryl exposure on the weight of evidence of those studies which are valid and interpretable.

Based on an assessment of the weight of currently available evidence, two general conclusions may be drawn concerning the potential of carbaryl to affect mammalian development. The first is that the administration of carbaryl to pregnant animals (at sufficiently high dose levels and/or sufficient duration of treatment) may result in adverse effects to the embryo or fetus. Of those studies from which definite conclusions may be drawn, carbaryl has been shown to produce terta in the guinea pig, rabbit, and dog; and fetoxicity in the mouse, rat, and gerbil. The second conclusion which may be arrived at is that these effects have generally occurred at dose levels which are toxic to the maternal animal. Adverse developmental effects have been seen at levels which resulted in maternal death in the guinea pig, cholinergic toxcity in the rabbit, and weight loss in the rat and mouse (the health status of the maternal gerbils was not given in the published study concerning that species). The dog appears to be the only exception to this conclusion, and in this species the treated females had difficulty giving birth, a possible sign of carbaryl-induced maternal toxicity. In the Agency's judgment, the quality of the studies by Smalley et al., and Imming et al., which were conducted more than a decade ago, does not meet current scientific standards. Insufficient numbers of animals were included in the dose groups, and insufficient attention was paid to the condition of the bitches throughout the period of dosing, and to maternal and fetal blood levels of the compound.

The Agency fully acknowledges that the evaluation of these studies in terms of their applicability to the human population must be done with great care. In assessing the totality of the experiments, the Agency recongnizes that carbaryl has been tested in an extremely wide variety of species and has been found to be teratogenic only in three species (guinea pig, rabbit, and dog), of which defects were found in only one species (the dog) at doses below those causing maternal toxicity. In view of the circumstance that there are adequate prenatal studies in eight species, it would appear that carbaryl is not a potent teratogen. This same close dose relationship exists between maternal toxicity and forms of adverse fetal effects other than teratogenicity.

In judging the relevance of embryonic fetal effects which are "confounded" by gross maternal toxicity, several factors must be kept in mind. In an experiment where significant differences are seen between control and treated groups, these differences are by definition attributable to treatment. If adverse perinatal effects are found in carbaryl-treated litters, the cause of these effects is understood to be carbaryl. Even if these effects are seen only in litters of mothers who were themselves adversely affected by the carbaryl treatment, we must still conclude that carbaryl is the cause of the developmental effects.

When the Agency begins to consider the relevance of such studies to the human population, however, other factors must be taken into account. Foremost are possible mechanisms by which the effect in the developing organism was obtained. If, for instance, a compound is administered which affects the maternal animal's desire to eat, the mother may lose weight and be unable to provide sufficient nourishment to either the fetal or neonatal animal. A study such as this raises the question, however, whether any type of food restriction would have resulted in the same effect. If this is the case, the primary effect would be the food restriction, and the mechanism for producing it would become almost incidental. In the study by Robens (1968), for example, carbaryl produced terata in guinea pigs at doses which resulted in 40% maternal mortality. While carbaryl was by definition the teratogenic agent, it is very possible that the actual cause of the terate lies in some part of the general debilitation of the surviving animals. Without a control group which is equally sick it is impossible, however, to distinguish between the unique effects of carbaryl and the effects of the severe maternal toxicity. In all the species tested, with the possible exception of the dog, adverse fetal effects were not seen when there was no maternal toxicity. There is a valid question, therefore, as to whether carbaryl itself has any properties which render it fetotoxic or whether carbaryl, like most pesticides, can cause severe maternal toxicity when administered at sufficiently high doses, and some aspect(s) of this toxicity in turn results in adverse developmental effects.

Another aspect of the relationship of maternal toxicity to adverse fetal effects is the relationship between the effect doses of both types of effects. It is generally felt that a compound which is fetotoxic at much lower dose levels than it is maternotoxic has the potential to be a greater human hazard than one in which the effect levels are similar. The reason for this is that the former case is far more insidious environmentally, since a slight rise in terata or other fetal toxicity is extremely difficult to identify by epidemiological means. Acute health problems in the adult population are easier to identify, and corrective action may be swiftly taken. The relationship between maternal and fetal toxic dose levels clearly approaches equality in most species tested with carbaryl. Even in the case of the dog, some signs of maternal toxicity occurred at doses where terata were noted, indicating that in this species, too, adverse developmental effects were not seen at doses below those which elicited maternal toxicity.

In view of the overall weight of evidence of studies which are valid and interpretable, the Agency has concluded that currently available data on carbaryl do not indicate that a rebuttable presumption on the basis of teratogenic and fetotoxic effects is warranted at this time. In the Agency's judgment, the extremely high doses of carbaryl used to elicit effects in the developing organism, coupled with the positive correlation of maternal and fetal toxicity in the multiple species tested (the dog being a possible exception), indicate that carbaryl would not constitute a potential human teratogenic or reproductive hazard under proper environmental usage.

2. *Mutagenicity.* 40 CFR 162.11(a)(3)(ii)(A) provides that "a rebuttable presumption shall arise if a pesticide's ingredient(s), metabolite(s), or degradation product(s) * * * induces mutagenic effects, as determined by multitest evidence." 40 CFR 162.3(y) defines mutagenicity as "the property of a substance or mixture of substances to induce changes in the genetic complement of either somatic or germinal tissue in subsequent generations."

The Agency has reviewed the extensive data which are currently available on the mutagenicity of carbaryl and conducted an assessment of the mutagenic potential of carbaryl based on the weight of existing evidence. Specifically, the primary objective of a mutagenicity risk assessment is to determine the potential of a chemical to cause heritable germline effects in man (Environmental Protection Agency's Proposed

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Guidelines for Mutagenicity Risk Assessments (45 FR 74984–74988). Only mutagenicity as a possible adverse effect of carbaryl per se is at issue in this determination. The possibility of a mutagenic risk posed by formation of N-nitrosocarbaryl in stomach physiology will be addressed in a separate Agency review of nitroso compounds.

In broad summary, carbaryl has been reported to produce gene mutations in bacteria, *Drosophila*, and mammalian cells in culture. However, there are several inadequacies in these studies. In addition, the results of cytogenetic tests imply that carbaryl may induce chromosomal effects in mammalian cells in culture, in whole mammals, and in plants, and carbaryl has been shown to cause primary DNA damage in cultured human cells. Collectively, all of these factors strongly suggest that carbaryl may act as a mutagen. To cause heritable effects in man, however, a chemical with intrinsic mutagenicity must reach the germinal tissue. Evidence that carbaryl and/or its active metabolites reaches the gonads is only suggestive. Gonadal effects in males—e.g., abnormal sperm morphology, reduction in the number of spermatogonia and spermatozoa in the seminiferous tubules, and reduced sperm motility—have been observed in rodents exposed to carbaryl (Degraeve et al., 1976; Shtenberg and Rybakava, 1968; Kitagawa et al., 1977). In addition, abnormal sperm head morphology has been reported in workers with known exposure to carbaryl. Therefore, given the weight of evidence, carbaryl may have the potential to act as a germ cell mutagen. It should be emphasized, however, that carbaryl is not intrinsically a potent mutagen in the reported studies, and probably acts as a weak mutagen only.

a. *Evidence Concerning Point (Gene) Mutations—Bacteria.* Originally, McCann et al. (1975) classified carbaryl as nonmutagenic in the *Salmonella*/microsome assay using four histidine-requiring strains. Metabolic activation did not augment the mutation frequency. Later, however, McCann and associates re-examined their data and conducted additional experiments at different concentrations of carbaryl to detect reversion at the histidine locus of *Salmonella typhimurium* TA 1535 (base-pair substitution sensitive strain). In the absence of metabolic activation they found that carbaryl appears to be weakly mutagenic.

The mutagenicity of carbaryl was also evaluated by Rashid (1978), employing five strains of *Salmonella typhimurium*. Rashid found that carbaryl produced a weak positive response only in strain TA 1535 (missense) in the absence of metabolic activation. The presence of rat liver (S–9) microsomal enzymes did not increase the reversion frequency. Cook (1977 [abstract]) observed weak mutagenic activity (2-fold increase) on another base-pair substitution sensitive strain TA 100 in the absence of metabolic activation. Egert and Greim (1976) reported positive mutagenic activity using the *Salmonella typhimurium* strain sensitive to frameshift mutagens TA 1538, with metabolic activation.

In contrast, several investigators have reported negative responses with carbaryl using the *Salmonella*/Ames test (Marshall et al., 1976; Shirasu et al., 1976; DeLorenzo et al., 1976; Blevins et al., 1977). Other bacterial tests in which carbaryl has been reported as negative include back mutations in *Bacillus subtilis* (DeGiovanni-Donnelly et al., 1976), forward mutations in *Haemophilus influenzae*. (Elespuru et al., 1974) and back and forward mutations in *Escherichia coli* (Ashwood-Smith et al., 1972; Egert and Greim, 1976; Fahrig, 1974; Ficsor and Ni Lo Piccolo, 1972; Nagy et al., 1975; Shirasu, et al., 1976).

b. *Evidence Concerning Point Mutations—Mammalian Cells in Culture.* Ahmed et al. (1977) found approximately a 9-fold increase in the number of ouabain-resistant (OUA^R) mutants over spontaneous mutants after V79 Chinese hamster cells were treated in monolayer with 10 uM of carbaryl (66% cell survival). These OUA^R mutants were reported to be phenotypically stable. Although carbaryl was found to be weakly mutagenic in this gene mutation assay, several inadequacies are apparent in this report which reduce the weight of the positive result: e.g., (1) a concentration-related increase in mutation frequency was not demonstrated, (2) concurrent positive controls were omitted, and (3) data were not presented to support the statement of phenotypic stability of the ouabain-resistant phenotype.

c. *Evidence Concerning Point Mutations—Drosophila: Sex-linked Recessive Lethal Test.* Brezheskii (1972) exposed *Drosophila melanogaster* males to a 1% suspension of "Sevin" (85% carbaryl) in dilute sugar for 24 hours (50% survival). Brezheskii reported a small increase in the percentage of complete (heritable mutations) and partial (not all mutations transmitted to progeny) recessive lethals (0.02%) as compared to control values.

d. *Evidence Concerning Primary DNA Damage.* Ahmed et al. (1977b) have shown that exposure of virally transformed human cells (VA–4) in culture to carbaryl initiates unscheduled DNA synthesis at exposure as low as 1 uM as determined (1) by autoradiography and (2) by photolysis of bromodeoxyuridine (BrdUrd), which is incorporated into DNA during DNA repair synthesis. Metabolic activation by liver microsomes did not enhance carbaryl's ability to induce unscheduled DNA synthesis. The cytotoxicity of the carbaryl doses used was not given in this report.

Regan et al. (1976) treated a culture of human skin cells with 100 uM of carbaryl for 1 hour and found no evidence of DNA damage. The technique employed, however, was not the same as that used by Ahmed et al. (1977b). Regan et al. (1976) determined the sedimentation profiles in alkaline sucrose gradients of cellular DNA treated with carbaryl as a detection method for DNA damage. This method of detection, however, may not be as sensitive as the (BrdUrd) photolysis method.

Siebert and Eisenbrand (1974) used a diploid strain of *Saccharomyces cerevisiae* D4 heteroallelic at the gene loci *ade*-2 and *try*-5 to assay for the ability of carbaryl to induce mitotic gene conversion in these loci. This is another assay that detects damage to DNA. In this organism, genetic activity (genetic damage) was not produced by a 16-hour carbaryl (1000 ppm) treatment. Yeast cells cultured in this solution showed only a 22% lethality.

e. *Evidence Concerning Chromosome Effects.* Several cytogenetic studies have shown that carbaryl can cause chromosome abnormalities (colchicine mitosis, chromosome lagging, chromosome fragmentation, multipolar anaphases, anaphase bridges, multinucleated cells) in both meiotic and mitotic chromosomes of plants (Wuu and Grant, 1966; Amer, 1965; Amer et al., 1971; Amer and Farah, 1968; Brankovan, 1972). Although carbaryl is capable of breaking chromosomes in plants, predominantly it causes mitotic disturbances by interfering with the spindle mechanism. This may result in chromosome loss and/or gain. Russian studies provide suggestive evidence that carbaryl may also act as an antimitotic agent in human cells in culture and in rats (Shpirt, 1975; Kazarnovskaya and Vasilos, 1977; Vasilos et al., 1972, 1975).

In addition, Ishidate and Odashima (1977) studied the effects of carbaryl on chromosomes of cultured Chinese hamster fibroblasts. Three different doses (0.0075, 0.015, 0.03 mg/ml) were added to cell cultures. At the maximum effective dose, 0.03 mg/ml (50% growth inhibition dose), several types of

chromosome aberrations (35% aberrant cells) were reported 48 hours after treatment. Specifically, chromatid gaps and breaks, chromosome breaks, translocation, ring formation, and fragmentation were observed with a higher frequency than in non-treated control cultures (1% aberrant cells). Although the authors stated the "gaps" were the predominant chromosomal effect, the frequency of occurrence for each particular type of aberration and the frequency of aberrations within a cell were not given. At lower doses, 0.015 mg/ml resulted in 24% aberrant cells, and 0.0075 did not appreciably affect chromosome structure (1% aberrant cells). The authors did not give the toxicity of these doses.

The dominant lethal assay in rodents, which detects chromosome damage in germ cells, was used both by Epstein et al. (1972) and by Weil et al. (1973). Using male mice, Epstein et al. (1972) administered 1000 mg/kg and 50 mg/kg (subtoxic doses) of carbaryl by gavage in daily portions over five consecutive days. Reportedly, this dosage schedule did not produce significant early fetal deaths or preimplementation losses. However, data were not presented in this report to support this statement. Weil et al. (1973) looked for dominant lethality in rats using a 3-generation study and found no significant lethal effects. The authors do not state if the carbaryl dosage level was the maximum-tolerated dose. Furthermore, the number of males treated, the number of virgin untreated females mated with each treated male, and the number of implant and fetal deaths per female of test or control groups are not given in this report.

f. *Evidence as to Whether Carbaryl Reaches the Germinal Tissue.* In order for any mutagen to cause genetic alterations that may be inherited by future generations, it must reach the gonads. Numerous inadequacies are apparent in the available reports concerning the potential of carbaryl to reach the mammalian gonads, and the evidence is considered suggestive rather than conclusive.

Two epidemiological studies of gonadal effects in carbaryl-exposed males have been reviewed by the Agency. Wyrobek et al. (1980) analyzed semen samples from 50 carbaryl production workers who had spent at least one year on the job, using semen from thirty-four new hires for control purposes. A significant elevation of sperm abnormalities (abnormal head morphology) in currently exposed workers as compared to controls was observed. However, previously exposed workers (an average of 6.5 years since last carbaryl exposure) did not exhibit a significant elevation of sperm abnormalities from control values. Because of the small sample size, however, it cannot be established if carbaryl effects are reversible. Although this study reports a statistical correlation between working in a carbaryl-exposed area and an alteration of human spermatozoa, it is not clear that carbaryl is the causative link since these workers were exposed to pesticides other than carbaryl. The Agency has serious doubts about the validity of this study in view of the generally weak methodology employed. A small number of employees was observed, no longitudinal follow-up (multiple sampling with the same workers) was carried out, study results were in all likelihood confounded by the effects of aging in view of the difference in mean age between new-hire controls (24 years) and exposed subjects (39 years), and those workers who were subject to the longest durations of exposure exhibited lesser sperm morphology effects. In an earlier epidemiological study performed with workers at the same plant studied by Wyrobek et al. (1980), Whorton and Milby (1978) examined semen samples provided by 47 workers. The results of the semen counts were compared to semen counts obtained from 90 members of a composite (off-site) control population. A higher percentage of the carbaryl-exposed workers (14.9%) showed depressed sperm counts (less than 20 million per ml) than did the control group (5.5%), but the difference is not considered statistically significant ($p=0.0686$). Reproductive hormone levels in the carbaryl-exposed workers were normal.

In addition to the epidemiological studies cited above, the Agency has reviewed a number of gonadal studies in rodents, though most of these reports are flawed in terms of the rigor of the experiments performed, or by inadequate presentation of data. The following studies reported adverse gonadal effects: Krylova and Denisova (1973), [Russian study]), Kitagawa et al. (1977), Degraeve et al. (1976), Thomas et al. (1974), and Shtenberg and Rybakova (1968). In contrast, some investigators have reported no significant gonadal effects attributable to carbaryl: Dikshith et al. (1976) and Weil et al. (1973).

Due to the weak mutagenic responses which have been measured, and due to the suggestive rather than conclusive nature of the evidence available as to the potential of carbaryl to reach the germinal tissue, the Agency has determined that the weight of evidence does not constitute a basis on which to conclude that carbaryl poses a mutagenic risk. The Agency has therefore determined that a rebuttable presumption against carbaryl on the basis of mutagenic effects is not warranted at this time.

3. *Oncogenic Effects.* 40 CFR 162.11(a)(3)(i)(A) provides that a rebuttable presumption shall arise if a pesticide's ingredient(s), metabolite(s), or degradation product(s) induce(s) "oncogenic effects in experimental mammalian species or in man as a result of oral, inhalation or dermal exposure." The term "oncogenic" is defined in 40 CFR 162.3(bb) as "the property of a substance or mixture of substances to produce benign or malignant tumor formation in living anaimals." To determine whether or not carbaryl might pose an oncogenic hazard warranting a rebuttable presumption, the Agency has conducted a review of the available literature. Only oncogenicity as a possible adverse effect of carbaryl per se is at issue in this determination. The possibility of an oncogenic risk posed by formation of N-nitrosocarbaryl in the stomach will be addressed in a separate Agency review of nitroso compounds.

Innes et al. (1969) administered 4.64 mg/kg carbaryl in 0.5% gelatin daily by stomach tube to mice, beginning at 7 days of age and continuing until mice were 6 weeks old. (Carbaryl dosage was not adjusted according to weight gain during the 6-week period.) Vehicle groups and untreated control groups consisted of 18 animals each. Subsequently, the vehicle groups were fed a diet containing 14 ppm carbaryl, and the experiment was terminated when the mice were approximately 78 weeks of age. Treated mice showed no significant increase in the incidence of tumors over controls. It should be pointed out, however, that the 14 ppm dietary dosage of carbaryl was probably too low to be a firm indicator of no oncogenic potential. In a parallel experiment, Innes et al. (1969) administered a single subcutaneous dose of 100 mg carbaryl/kg in DMSO to weanling mice. Tumor incidence in treated animals was not significantly increased.

In a study conducted by the Mellon Institute (1963), groups of 48 male and 48 female CD–1 mice were fed diets containing 0.04%, 0.01%, and 0% carbaryl. Seventy-two of the 288 animals included in the study were given gross and histopathological examination. Of the other 216 animals, those which died during the initial 80-week period (50%) were subjected to gross examination.

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and tissues suspected of having pathology were examined histologically. Of the 65 animals allowed to live for 2 years, 9 males and 21 females were sacrificed at the end of the period, and no information was given on the remaining animals. No significant difference in tumor incidence between treated and untreated animals was observed in those mice sacrificed after 80 weeks, those which died during the 80 week period, or those sacrificed at the end of 2 years. Because this study is very seriously flawed, however, it cannot stand as a basis for any determination for or against the carcinogenic potential of carbaryl. Among numerous drawbacks, the most serious problems are (1) that no information is available concerning 93 of the 288 animals with which the experiment began, and (2) that for animals dying during the study, histopathology was carried out only on animals suspected of tumor growth and not on all animals.

In another experiment conducted by the Mellon Institute (1962), a mixture of 40 parts Sevin 85 sprayable powder (85% carbaryl) and 60 parts water was applied (schedule of application unspecified) on the skin of 36 mice for 30 months. None of the animals developed tumors. In the same experiment, methlycholanthrene (0.2% solution in acetone) was applied to a group of 32 animals. All animals in this group developed tumors by the end of 12 months. Details concerning pathology and experimental procedure were not available for review, however.

Makovskaia et al. (1965) administered weekly doses of carbaryl (60 mg/kg in a 2% solution in sunflower oil) intraperitoneally to groups of line A and C_3HA mice. The experiment included 400 carbaryl-treated animals, 150 animals treated with urethane (200 to 1000 mg/kg intraperitoneally) as a positive control group and 100 animals in the untreated control group. Animals were sacrificed at 1, 3, 5, 9, 12, 15, 18, and 24 months after the test began, and several organs (lungs, kidneys, liver, heart, spleen, pancreas, thyroid, and adrenals) were reportedly examined histopathologically. Makovskaia et al. reported that "carbaryl treatment did not produce any new growth in lungs or liver," but their report failed to provide experimental details necessary for evaluation. No conclusion concerning the carcinogenic potential of carbaryl can be drawn from this study.

Carpenter et al. (1961) maintained groups of male and female CF–N rats on a mixture of Purina Chow and carbryl (in concentrations of 0.04%, 0.02%, 0.01%, 0.005%, and 0.00%) for two years. Rats were 60 days old when the experiment began, and 20 rats were included in each group. Sixty-one of the 102 animals which died during the study, and 98 animals sacrificed at the end of the study, were examined histopathologically. Although female rats were found to have more pituitary tumors relative to males, no significant increase in tumors was found in treated groups relative to controls. The validity of Carpenter et al. is compromised, however, in that the study began not with newborn or weanling rats but with 60-day-old animals. Because they are known to be more sensitive to chemical carcinogenesis than are adults, newborn or weanling animals are preferred for purposes of cancer bioassays (CAG, 1977).

An an experiment conducted by Andrianova and Alekseev, a group of 60 adult male rats (mongrels) was given 30 mg/kg carbaryl orally (as a water suspension) twice weekly by gavage for 22 months. This dose was approximately one-twentieth of the LD_{50} dose. Tumors, as well as organs suspected of having tumors, were examined histopathologically. Out of 12 rats alive in the treated group at the end of the experiment, four animals were found to have malignant tumors (sarcomas). In another experiment, Androanova and Alekseev (1970) introduced 20 mg of carbaryl (enclosed in a purified paraffin capsule weighing 250 mg) hypodermically in a group of 48 male rats. Tumors were discovered in 2 out of 10 rats that survived 22 months. In both animals, tumors (diagnosed as fibrosarcoma) were observed under the skin near the back, not near the site of the implant. Whether or not controls were similarly implanted with gelatin capsules without the chemical was unclear. Andrianova and Alekseev used a group of 48 untreated rats as controls for both these experiments. Among controls, one fibrosarcoma was discovered after 11 months, at which time 46 of the original controls remained alive. The number of controls surviving the full 22 months of the experiment was not given.

In a study performed by Shimkin et al. (1969), the carcinogenic potential of carbaryl was tested with the pulmonary tumor response in male A/He mice, 7 to 9 weeks old. Carbaryl was prepared by reacting its chloroformate with ammonia. The dosing vehicle was 99.9% pure tricaprylin. Intraperitoneal injections of 20 mg carbaryl/kg were made 3 times weekly for 4 weeks. The mice were sacrificed at 20 weeks after the final injection. Necropsies were performed, and lungs were examined microscopically. Untreated and vehicle-treated control animals were similarly maintained and evaluated. Fifteen of an original 16 carbaryl-treated animals survived to 20 weeks. Upon sacrifice and examination, one lung tumor was found in each of 4 animals, and 2 lung tumors were found in each of 2 animals. Twenty-eight of an original 32 vehicle control animals survived to be sacrificed. Upon examination, 1 lung tumor was found in each of 6 animals, and 1 animal had 2 tumors. Thirty-one of an original 32 untreated control animals survived to be sacrificed. Upon examination, 1 lung tumor was found in each of 2 animals. The number of lung tumors per mouse in the carbaryl-treated, vehicle control, and untreated control groups was, respectively, 0.7, 0.3 and 0.1.

The tumor incidence observed by Shimkin et al. in carbaryl-treated mice (40%), was slightly higher than in the vehicle control group (25%). However, this difference is not statistically significant. Moreover, Shimkin et al. in fact state that a slightly higher tumor incidence in vehicle-control mice (used as the standard comparison group in the above calculation) over untreated control mice is commonly observed in this strain. In the Agency's judgment, no conclusion about the potential human carcinogenicity of carbaryl can be made from this test.

A study has been reported by A. J. Triolo (1978) in a U.S. EPA document, which attempted to evaluate the ability of carbaryl to enhance the incidence of forestomach tomors in Ha/ICR female mice and lung tumors in A/J female mice treated with benzo(a)pyrene (BP). Dietary levels of carbaryl up to 2000 ppm did not increase the incidence of forestomach tumors in mice treated with 300 ppm benzo(a)pyrene in the diet for 12 weeks. In A/J mice given 3 mg benzo(a)pyrene *per os* on days 7 and 2[illegible] 1000 ppm carbaryl in the diet produced statistically significant ($P<0.05$) increase in lung tumor incidence (18/18 as compared to the BP-treated control (15/17). This increase is slight, however and in a repeat experiment the incidence in the carbaryl-BP group (16/34) was similar to that in the BP group (16/31). The A/J mice were on study for 20 and 16 weeks, respectively, in these experiments. The Agency's conclusion with respect to this study is that a co-carcinogenic effect has not been conclusively demonstrated.

Based on the weight of evidence of available studies on carbaryl the Agency has concluded that a rebuttab[illegible]

presumption on the basis of oncogenic effects is not warranted a this time.

4. *Neurotoxicity.* As stipulated in 40 CFR 162.11(a)(3)(ii)(B), "a rebuttable presumption shall arise if a pesticide's ingredient(s), metabolite(s), or degradation product(s) * * * produces any other chronic or delayed toxic effect in test animals at any dosdage up to a level, as determined by the Administrator, which is substantially higher than that to which humans can reasonably be anticipated to be exposed, taking into account ample margins of safety."

Three available studies concerning the neurotoxicity of carbaryl have been reviewed by the Agency. To test the neuromuscular degenerative potential of carbaryl as compared with that of triorthocresyl phosphate (TOCP), Carpenter et al. (1961) administered single subcutaneous injections (concentrations of 25% to 40% in lard) to 2-year-old moulting Rhode Island Red hens (13 and 10 hens, respectively). In addition, undiluted TOCP was given to one chicken, and undiluted lard was given to two others. Five control chickens received no injections. No adverse effects were observed at 1.0 g/kg or lower doses of carbaryl. Chickens administered 2.0 g/kg carbaryl showed leg weakness on the first or second day following dosing, and in only one case was the chicken nonambulant for 3 days. A nephrotoxic action was also observed in fowl which received 2.0 g/kg or larger injections of carbaryl. Necrosis was not present. In hens which received 3.0 g/kg carbaryl, leisions were observed. Such lesions were found at all levels in hens examined after injection with TOCP, with the anomalous exception of the 2.0 g/kg dosage. Likewise at all dosage levels tested, animals injected with TOCP showed fatty deposition of a similar though more diffuse nature to that observed in fowl administered 2.0 g/kg or larger doses of carbaryl. At 3.0 g/kg, TOCP proved lethal in 3 to 10 days, with leg weaknesses observed in 3 of 4 cases only on the day of death. At all lower dosage levels, weakness was not apparent until the thirteen or fourteenth day. Upon histopathological examination, slight evidence of demyelination was observed in three of the 10 TOCP-treated hens, whereas carbaryl-treated hens showed no signs of demyelination.

In screening tests for the production of paralytic effect in chicken hens, Gaines (1969) tested 30 organic phosphorous pesticides and 9 carbamate pesticides including carbaryl. Gaines (1969) administered carbarly subcutaneously to hens at doses of 800 and 1600 mg/kg and observed them for 24 days. Prior to carbaryl treatment, the hens were treated with 15 mg/kg of atropine orally. The hens treated with 1600 mg/kg dose of carbarly showed leg weakness within 24 hours, but all chickens recovered by day 24. The TOCP-treated animals developed paralysis after 14 days, and the paralysis continued until death.

Smalley et al. (1969) administered carbaryl at a dose of 150 mg daily for 72 and 83 days to one male pig and one female pig, respectively. In a second experiment, one female and two males given 150 mg/kg of carbaryl for 28 days, followed by 300 mg/kg/day for either 18 (males) or 57 (females) additional days. Ataxia was observed in both experiments. Microscopic examination of skeletal muscle showed myodegeneration. The author reported that hydrochlorothiazide, a diuretic, reversed the signs of toxicity of carbaryl administration in chronic testing of pigs.

Based on this currently available evidence, the Agency has concluded that carbaryl does not pose a neurotoxic hazard and that a rebuttable presumption on the basis of neurotoxicity is not warranted at this time.

5. *Viral Enhancement.* A recent study (Abrahamsen and Jerkofsky, 1980) which has come to the Agency's attention concerns viral enhancement as a possible adverse effect of human exposure to carbaryl. Abrahamsen and Jerkofsky investigated the effect of Sevin 4 oil on the replication of the human herpes virus varicella-zoster (VZ) in primary human embryonic lung (HEL) and HEP-2 cell cultures. Complete Sevin 4 oil, its active ingredient (analytic grade carbaryl) and its "base oil plus inert ingredients" in sub-toxic concentrations, were tested for the ability to enhace the growth of VZ virus as measured by an infectious center assay. A 12- to 15-fold increase in virus production by cells pretreated with Sevin 4 oil and carbaryl was observed. No enhancement was observed in base oil-treated cultures. Similar results were obtained when HEP-2 cells were used. Viral enhancement appeared to be concentration-related in that decreasing concentrations of carbaryl brought decreases in viral enhancement. Experiments with herpes simplex virus type 1, however, showed no viral enhancement by sevin 4 oil of any of its components. Abrahamsen and Jerkofsky suggest that the results of their work may be pertinent to studies of Reye's syndrome "since published epidemiological evidence has suggested a possible relationship between pesticide spraying, certain viral diseases including chickenpox or varicella, and the subsequent occurrence of Reye's syndrome" (Abrahamsen and Jerkofsky, 1980 [abstract]).

With regard to viral enhancement as a possible adverse effect of exposure to carbaryl, the Agency's determination at this juncture is that the work of Abrahamsen and Jerkofsky is preliminary in nature and that current data do not constitute a basis on which to conclude that carbaryl poses a human hazard in terms of viral enhancement. The Agency has therefore concluded that a rebuttable presumption is not warranted at this time.

B. Regulatory Determinations

As discussed in the preceding sections, the Agency has considered all available information on the human risks posed by the use of carbaryl and has concluded that the overall weight of evidence does not raise prudent concerns of unreasonable adverse risk. Consequently, the Agency has determined that the issuance of a rebuttable presumption against registration for carbaryl is not warranted at this time. Accordingly the Agency has determined to return carbaryl to the registration process. The reasons which form the basis for this determination are set forth in further detail in a separate Decision Document. Should further review of data call these determinations into question, the Agency will re-evaluate its conclusions and, if warranted, initiate an RPAR proceeding or take other appropriate regulatory actions.

C. Other Determinations

While the Agency has determined not to proceed with an RPAR action at this time, the Agency will pursue the courses of action outside the RPAR process to remedy data limitations and to ensure that exposure to carbaryl is held to reasonable levels.

During the review of carbaryl to determine if the available data indicated that the pesticide met or exceeded the RPAR risk criteria, the Agency discovered limitations in the data pertaining to the possible toxic effects of carbaryl. The Agency has therefore determined that it will request additional data, under the authority of Sec. 3(c)(2)(B) of FIFRA, to maintain the registrations of carbaryl products in effect. The request for this data is independent of the Agency's determination not to initiate a rebuttable presumption against registration for carbaryl pesticide products. The data requirements imposed by the Agency pursuant to Sec.

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3(c)(2)(B) will be formally communicated to the registrants in the near future. In addition, negotiations between the Agency and registrants will be initiated regarding appropriate label changes to ensure that exposure to carbaryl is held to reasonable levels.

IV. Procedural Matters

In accordance with the determinations announced in unit III of this Notice, the Agency will not initiate a rebuttable presumption against registration for pesticide products containing carbaryl at this time, but will return carbaryl to the registration process. Copies of this Notice and the separate Decision Document will also be transmitted to the affected registrants and applicants. Other interested persons may obtain a copy of the Decision Document by contacting Juanita Wills at the address or telephone number given in this Notice.

Upon receipt of the 3(c)(2)(B) data, and any other data which provide information about the potential of carbaryl to produce adverse effects in man and the environment, the Agency will review the carbaryl data base to determine if the initiation of an RPAR proceeding, or other appropriate regulatory action, is warranted.

Dated: December 14, 1980.

Edwin L. Johnson,

Deputy Assistant Administrator for Pesticide Programs.

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