Registration Decision RD2012-12

Acibenzolar-S-Methyl

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Registration Decision for Acibenzolar-S-Methyl

Health Canada's Pest Management Regulatory Agency (PMRA), under the authority of the *Pest Control Products Act*, and Regulations, is granting full registration for the sale and use of Acibenzolar-S-Methyl Technical and Actigard 50WG, containing the technical grade active ingredient acibenzolar-S-methyl, to control or suppress a variety of fungal diseases in tomato and tobacco.

An evaluation of available scientific information found that, under the approved conditions of use, the product has value and does not present an unacceptable risk to human health or the environment.

These products were first proposed for registration in the consultation document[1] Proposed Registration Decision PRD2010-19, *Acibenzolar-S-Methyl*. This Registration Decision[2] describes this stage of the PMRA's regulatory process for acibenzolar-S-methyl and summarizes the Agency's decision, the reasons for it and provides, in Appendix I, a summary of comments received during the consultation process as well as the PMRA's response to these comments. This decision is consistent with the proposed registration decision stated in PRD2010-19.

For more details on the information presented in this Registration Decision, please refer to PRD2010-19, which contains a detailed evaluation of the information submitted in support of this registration.

What Does Health Canada Consider When Making a Registration Decision?

The key objective of the *Pest Control Products Act* is to prevent unacceptable risks to people and the environment from the use of pest control products. Health or environmental risk is considered acceptable[3] if there is reasonable certainty that no harm to human health, future generations or the environment will result from use or exposure to the product under its conditions of registration. The Act also requires that products have value[4] when used according to label directions. Conditions of registration may include special precautionary measures on the product label to further reduce risk.

1 "Consultation statement" as required by subsection 28(2) of the *Pest Control Products Act.*

2 "Decision statement" as required by subsection 28(5) of the *Pest Control Products Act.*

3 "Acceptable risks" as defined by subsection 2(2) of *Pest Control Products Act.*

4 "Value" as defined by subsection 2(1) of *Pest Control Products Act*"...the product's actual or potential contribution to pest management, taking into account its conditions or proposed conditions of registration, and includes the product's (*a*) efficacy; (*b*) effect on host organisms in connection with which it is intended to be used; and (*c*) health, safety and environmental benefits and social and economic impact".

To reach its decisions, the PMRA applies modern, rigorous risk-assessment methods and policies. These methods consider the unique characteristics of sensitive subpopulations in humans (for example, children) as well as organisms in the environment (for example, those most sensitive to environmental contaminants). These methods and policies also consider the nature of the effects observed and the uncertainties when predicting the impact of pesticides. For more information on how the PMRA regulates pesticides, the assessment process and risk-reduction programs, please visit the Pesticides and Pest Management portion of Health Canada's website at www.healthcanada.gc.ca/pmra.

What Is Acibenzolar-S-Methyl?

Acibenzolar-S-methyl is the active ingredient in the end-product Actigard 50WG, which is formulated as a water–dispersible granule. It controls or suppresses disease by inducing the host plant's defence responses. Actigard 50WG is to be used for suppression of bacterial spot (*Xanthomonas campestris* pv. *vesicatoria*) and bacterial speck (*Pseudomonas syringae* pv. *tomato*) on tomato and for control of blue mold (*Peronospora tabacina*) on tobacco.

Health Considerations

Can Approved Uses of Acibenzolar-S-Methyl Affect Human Health?

Acibenzolar-S-methyl is unlikely to affect your health when used according to label directions.

Potential exposure to acibenzolar-S-methyl may occur through the diet (food and water) or when handling and applying the product. When assessing health risks, two key factors are considered: the levels where no health effects occur and the levels to which people may be exposed. The dose levels used to assess risks are established to protect the most sensitive human population (for example, children and nursing mothers). Only uses for which the exposure is well below levels that cause no adverse effects in laboratory animals are considered acceptable for registration.

Toxicology studies in laboratory animals describe potential health effects from varying levels of exposure to a chemical and identify the dose where no adverse effects are observed. The health effects noted in animals occur at doses more than 100-times higher (and often much higher) than levels to which humans are normally exposed when acibenzolar-S-methyl products are used according to label directions.

In laboratory animals, the acute toxicity of acibenzolar-S-methyl was low by the oral, dermal and inhalation routes of exposure. It was minimally irritating to the eyes and skin, but is considered a potential skin sensitizer. Consequently, the hazard signal words "POTENTIAL SKIN SENSITIZER" are required on the label. Similarly, in laboratory animals the acute toxicity of the end-use product Actigard 50WG was low by the oral, dermal and inhalation routes of exposure. It was minimally irritating to the eyes, but moderately irritating to the skin, and is not considered a skin sensitizer. Consequently, the hazard signal words "WARNING – SKIN IRRITANT" are required on the label.

Acibenzolar-S-methyl did not cause cancer in animals and was not genotoxic. There were also no effects on reproduction. The first signs of toxicity in adult animals given daily doses of acibenzolar-S-methyl over longer periods of time were spleen effects in the mouse. At higher doses, or with longer periods of exposure, toxicity effects occurred in the blood, liver, spleen and bone marrow in mice, rats and dogs. Body weight effects also occurred in these three species, as well as in the rabbit. There was no indication that acibenzolar-S-methyl caused damage to the adult nervous system. However, acibenzolar-S-methyl given to pregnant animals resulted in changes in brain development, as well as birth defects, at doses that were not toxic to the mother. The developing foetus and early postnatal young are potentially more sensitive to acibenzolar-S-methyl than adult animals. As a consequence, extra factors were applied during the risk assessment to further reduce the allowable level of human exposure to acibenzolar-S-methyl. The risk assessment protects against these effects by ensuring that the level of human exposure is well below the lowest dose at which these effects occurred in animal tests.

Residues in Water and Food

Dietary risks from food and water are not of concern

Aggregate dietary intake estimates (food plus water) revealed that the general population and infants (<1 year old), the subpopulation which would ingest the most acibenzolar-S-methyl relative to body weight, are expected to be exposed to less than 9% of the acceptable daily intake. Based on these estimates, the chronic dietary risk from acibenzolar-S-methyl is not of concern for all population sub-groups. Acibenzolar-S-methyl is not carcinogenic, therefore, a chronic cancer dietary risk assessment is not required.

An aggregate (food plus water) dietary intake estimate for the highest exposed population (children 1-2 years old) was less than 94% of the acute reference dose, which is below the level of concern. Therefore, the acute dietary risk from acibenzolar-S-methyl is below the level of concern for all population sub-groups.

The *Food and Drugs Act* prohibits the sale of adulterated food, that is, food containing a pesticide residue that exceeds the established maximum residue limit (MRL). Pesticide MRLs are established for *Food and Drugs Act* purposes through the evaluation of scientific data under the *Pest Control Products Act (PCPA).* Food containing a pesticide residue that does not exceed the established MRL does not pose an unacceptable health risk.

Residue trials conducted throughout the United States using acibenzolar-S-methyl on tomatoes and tobacco were acceptable to support the domestic uses. No new MRLs are recommended at this time as MRLs for tomatoes and tomato paste were previously established to cover residues in imported commodities. The MRLs for this active ingredient can be found in the Science Evaluation section of PRD2010-19.

Occupational Risks From Handling Actigard 50WG

Occupational risks are not of concern when Actigard 50WG is used according to the label directions, which include protective measures.

Farmers and custom applicators who mix, load or apply Actigard 50WG as well as field workers re-entering freshly treated fields can come in direct contact with Actigard 50WG residues on the skin. Therefore, the label specifies that anyone mixing/loading and applying Actigard 50WG must wear a long-sleeved shirt, long pants, and shoes or boots with socks, and may not treat more than 100 hectares in a day. In addition, workers mixing and loading the concentrated product must wear chemical resistant gloves and goggles. The label also requires that workers do not enter treated fields for 12 hours after application to tomatoes and for 8 days after application to tobacco. Taking into consideration these label statements, the number of applications and the expectation of the exposure period for handlers and workers, the risk to these individuals are not a concern.

For bystanders, exposure is expected to be much less than that for workers and is considered negligible. Therefore, health risks to bystanders are not of concern.

Environmental Considerations

What Happens When Acibenzolar-S-Methyl Is Introduced Into the Environment?

Environmental risks are not of concern.

Acibenzolar-S-methyl is degraded by both chemical reactions and microorganisms in soil and water. Acibenzolar-S-methyl is non-persistent in soil and in aquatic systems. Transformation in water is enhanced by sunlight, but not in soil. The major transformation product of acibenzolar-S-methyl, CGA 210007, is mobile in soil. An assessment of the leaching of CGA 210007 was conducted, and is dependent on many different factors. The assessment concluded that the concerns for CGA 210007 to leach to groundwater are minimal. Acibenzolar-S-methyl and CGA 210007 do not bioconcentrate and are therefore unlikely to bioaccumulate.

Although acibenzolar-S-methyl is highly toxic to aquatic organisms, adverse effects on these organisms through spray drift deposition in aquatic habitats adjacent to the treatment areas and at the application rates, are unlikely. There are no environmental risk concerns for acibenzolar-S-methyl or its transformation products affecting non-target terrestrial organisms as a result of spray drift in areas adjacent to the treatment area, at the application rates.

Value Considerations

What Is the Value of Actigard 50WG

Acibenzolar-S-methyl, the active ingredient in Actigard 50WG, suppresses bacterial spot (*Xanthomonas campestris* pv. *vesicatoria*) and bacterial speck (*Pseudomonas syringae* pv. *tomato*) on tomato and controls blue mold (*Peronospora tabacina*) on tobacco.

Acibenzolar-S-methyl has been identified as an important active for Canadian growers for use on several crops including apple, artichoke, basil, broccoli, cabbage, cucumber, lettuce, pepper, beans, strawberry, tomato, potato, pumpkin, squash and spinach.

The registration of Actigard 50WG on tomatoes and tobacco will provide growers with a different mode of action that would help to manage diseases on these crops. Currently, only two actives are registered for bacterial spot and bacterial speck on tomatoes while four actives are registered for blue mold control in tobacco.

Measures to Minimize Risk

Registered pesticide product labels include specific instructions for use. Directions include risk-reduction measures to protect human and environmental health. These directions must be followed by law.

The key risk-reduction measures on the label of Actigard 50WG to address the potential risks identified in this assessment are as follows:

Key Risk-Reduction Measures

Human Health

As there is a concern with users coming into direct contact with Actigard 50WG on the skin, anyone mixing, loading and applying Actigard 50WG must wear a long-sleeved shirt, long pants, and shoes or boots with socks, and may not treat more than 100 hectares in a day. In addition, workers mixing and loading the concentrated product must wear chemical resistant gloves and goggles. The label also requires that workers do not enter treated fields for 12 hours after application for tomatoes and for 8 days after application for tobacco. Standard label statements to protect against drift during application were added to the label.

Other Information

The relevant test data on which the decision is based (as referenced in PRD2010-19, Acibenzolar-S-Methyl) are available for public inspection, upon application, in the PMRA's Reading Room (located in Ottawa). For more information, please contact the PMRA's Pest Management Information Service by phone (1-800-267-6315) or by e-mail (pmra.infoserv@hc-sc.gc.ca).

Any person may file a notice of objection[5] regarding this registration decision within 60 days from the date of publication of this Registration Decision. For more information regarding the basis for objecting (which must be based on scientific grounds), please refer to the Pesticides and Pest Management portion of Health Canada's website (Request a Reconsideration of Decision, www.hc-sc.gc.ca/cps-spc/pest/part/protect-proteger/publi-regist/index-eng.php#rrd) or contact the PMRA's Pest Management Information Service.

[5] As per subsection 35(1) of the *Pest Control Products Act.*

Appendix I Comments and Responses

1. Comments on rat developmental toxicity with respect to the oral and dermal exposure routes as well as developmental neurotoxicity were received and assessed with the following responses:

Oral Exposure Route

Comment 1: It was commented that a sufficient weight of evidence has been provided to the PMRA to support the view that "the incidences of umbilical hernia observed at 10 mg/kg in the original rat developmental toxicity study are not related to treatment". Briefly, this weight of evidence consists of: i) "no other malformations or variations at 10 mg/kg", ii) "no malformations at 50 mg/kg", iii) "no umbilical hernias at higher doses" (i.e. no dose response), iv) "no umbilical hernias were observed in additional guideline and exploratory studies of rat developmental toxicity" and v) "historical control data indicated a similar incidence of this finding in untreated rats of the same strain".

As there is no evidence of developmental toxicity, the United States Environmental Protection Agency has assigned a NOAEL of 50 mg/kg; therefore, the PCPA factor may be reduced to 1. The acute and chronic reference dose levels, and the occupational exposure endpoints may be adjusted to reflect a standard uncertainty factor of 100.

Response to Comment 1: With specific reference to the summary points that constitute the weight of evidence provided under Comment 1, the PMRA considers that the midline closure defect, umbilical hernias, which occurred at 10 mg/kg bw/d, cannot be ruled out as being treatment-related based on: i) the occurrence of additional developmental effects in other studies at essentially the same dose level or higher. These developmental effects included morphometric changes in the cerebellum and the dorsal cortex at 8.2 mg/kg bw in the rat developmental neurotoxicity (DNT) study and malformations at greater than the spontaneous incidence rate at 10 and 75 mg/kg bw in a second oral rat developmental toxicity study. Other factors are: ii) the understanding that, for rare malformations, neither a clear dose response, nor a positive occurrence at all dose levels is a realistic experimental outcome given the small number of litters examined per treatment group; iii) the occurrence of other midline closure defects, including omphalocele, gastroschisis and craniorachishisis, at higher dose levels, iv) the occurrence of one additional umbilical hernia (range-finding rat developmental toxicity study, 250 mg/kg bw/d, PMRA#1586953) among the 54 litters examined in other guideline and exploratory studies that used comparable dosing conditions and the same strain of rat, v) a total incidence of umbilical hernias among treated rats, from all comparably conducted developmental toxicity studies (2.27% of litters, 3/132 treated litters examined), that exceeded the spontaneous incidence rate for this strain of rat (0.77% of litters, 6/784 control litters).

As such, a PCPA factor of 10-fold was retained, as outlined in PRD2010-19 under Section 3.1.1., because of an increased susceptibility of the young to developmental toxicity effects, noted in the DNT study as well as the oral developmental toxicity study, that were considered serious and occurred in the absence of maternal toxicity.

Dermal Exposure Route

Comment 2: With reference to paragraph 2 on page 14 of the proposed regulatory decision (PRD2010-19), the PMRA has incorrectly concluded that a dermally applied dose of 500 mg/kg is equivalent to an oral dose of no greater than 0.65 mg/kg bw/d. This conclusion is not supported by data from the dermal metabolism study. In this study, the dermal penetration rate at the high dose was nearly constant, exhibiting steady state conditions for 24 h. At the low dose, steady state conditions were not evident; the dermal penetration rate was maximal at 4 h (0.81 $\mu g/cm^2/h$) and declined thereafter over the 24 h exposure period. Thus, dermal penetration rates can be dose and time dependent. Similarly, percent absorption, which is a different measure than the dermal penetration rate, was also dose and time dependent. Given that the amount of acibenzolar-S-methyl that is absorbed dermally is clearly dependent on the applied dose and duration of the exposure, the PMRA's conclusion of an oral equivalent dose of 0.65 mg/kg is incorrect. In addition, differences in experimental conditions between the dermal metabolism study and the dermal developmental toxicity study (for example, vehicle used) make such a direct comparison of dose equivalence difficult.

Response to Comment 2: Comparison of the adverse health effects that occur with oral versus dermal exposure can provide insight into route-specific toxicity. Since this comparative evaluation is most meaningful at an equivalent internal dose, the PMRA relied on the dermal metabolism study to facilitate this assessment. The PMRA agrees that the amount of acibenzolar-S-methyl that is absorbed dermally depends on both the dose and duration of the exposure. In addition, the amount absorbed depends on the size of the area of exposure and on whether the applied dose exceeds the skin's intrinsic capacity to transport acibenzolar-S-methyl. When this transport capacity is exceeded, the underlying transport processes become saturated; the penetration rate and the amount absorbed are no longer dependent on the dose applied. When saturation occurs, an increase in the amount of acibenzolar-S-methyl applied to a localized area of skin does not result in a corresponding increase in its *rate* of transport across the skin. Under these conditions, a chemical's dermal penetration rate is considered *maximal*; near-steady state (i.e. approximately constant) transport characteristics will occur transiently, as long as the saturation persists and elimination processes at the site of uptake are sufficient to minimize local accumulation within the skin. Thus, the initial rate of dermal penetration and the amount absorbed will increase as the dose increases, but only under conditions that do not saturate the processes responsible for transporting acibenzolar-S-methyl across the skin.

A reliable way to characterize the dose-dependence of dermal penetration, and to establish whether chemical transport has been saturated by the dose applied, is to estimate the *initial* rate of transport. For acibenzolar-S-methyl the initial dermal penetration rate estimates, at dose levels of 0.53 mg/kg bw (11.2 $\mu g/cm^2$, low dose) and 4.51 mg/kg bw (103.7 $\mu g/cm^2$, high dose), were 0.77 and 0.78 $\mu g/cm^2/h$, respectively. Thus, a 10-fold increase in the applied dose of acibenzolar-S-methyl resulted in no increase in its initial rate of transport across the skin. This indicates that, at both of the doses tested in the rat dermal metabolism study, the initial rate of dermal penetration was maximal and the underlying transport processes were fully saturated. Based on direct quantification of the total amount of acibenzolar-S-methyl that was absorbed, saturation of transport occurred for at least 4 h at the low dose; the resulting dermal penetration rate estimate for this period of time was 0.81 $\mu g/cm^2/h$. This rate estimate is effectively the same as the average initial rate estimate (0.78 $\mu g/cm^2/h$) of the two dose levels tested. As long as dose saturation persists, this average initial rate can provide a reliable estimate of the amount of

acibenzolar-S-methyl that is absorbed for any duration of exposure, regardless of the dose. For instance, in the dermal metabolism study 3, 6 and 16% of the high dose was absorbed over the 4, 8 and 24 h exposure periods, respectively; values of 3, 6 and 18%, respectively, were calculated using the average initial rate estimate. One can also calculate that 12.8 μg/cm^2 is the lowest dermal dose required to maintain saturated transport conditions for 6 h, the duration of exposure used in the dermal developmental toxicity study.

In the dermal developmental toxicity study, rats receiving 10, 100 and 500 mg/kg bw/d had dermal doses of ~ 59, 601 and 2,936 μg/cm^2 of skin, respectively. All of these dose levels are expected to saturate the dermal penetration processes for acibenzolar-S-methyl for the entire 6 h exposure; the lowest dose is nearly 5-fold greater than the minimal dose required to achieve this. As a consequence of dose saturation, the maximal amount of acibenzolar-S-methyl absorbed through the skin in the dermal developmental toxicity study is not expected to be greater than ~ 0.65 mg/kg bw/d, regardless of the dose applied. This was calculated by multiplying the maximal dermal penetration rate (0.78 μg/cm^2/h) by the exposure duration (6 h/d) and the surface area of the application site (~ 41 cm^2) in the dermal developmental toxicity study. The resulting estimate of total daily dermal absorption (~ 192 μg/d) was normalized to the time-averaged body weights (~297 g) of the treated animals in this study. The resulting value was considered equivalent to an orally administered dose because animals gavaged with up to 100 mg/kg bw in the oral metabolism study absorbed at least 91 to 96% of the dose. If 0.65 mg/kg bw/d was given orally, by gavage, essentially all of it would be absorbed.

The PMRA agrees that differences in experimental conditions between the dermal metabolism study and the dermal developmental toxicity study may make it difficult to directly compare the dose equivalence between these studies. The only potentially substantive differences between these two studies were the vehicle used and the sex of the animals. Although the Lot # of acibenzolar-S-methyl used was also different, the stated purity differed by no more than 1.1%. Both studies were conducted by the same laboratory within a one-year time-frame using essentially the same housing and husbandry conditions, the same diet, and animals from the same supplier which were the same age, strain, approximate weight. Although the vehicle used in the two studies was different, absorption through the skin was likely greater in the dermal metabolism study than in the dermal developmental toxicity study because of the physicochemical characteristics of the vehicles chosen and the low aqueous solubility of acibenzolar-S-methyl. As a result, the estimated initial penetration rate from the dermal metabolism study is considered to have overestimated the dose potentially absorbed in the dermal developmental toxicity study. Finally, as previously noted, the lowest dose in the dermal developmental toxicity study is ~ 5-fold greater than the lowest dose required to saturate acibenzolar-S-methyl transport across the skin in the dermal metabolism study. Minimally, the transport capacity of skin in the rat developmental toxicity study would need to be 5-fold greater for any differences between these studies to have invalidated the estimated oral equivalent dose of 0.65 mg/kg bw/d. Thus, comparative insight into the route-specific developmental *toxicity* of acibenzolar-S-methyl is limited because the highest estimated internal dose in the dermal developmental toxicity study is well-below the lowest dose that results in developmentally toxic effects via the oral route; an equivalent internal dose was not achieved.

Comment 3: The final sentence in paragraph 2 on page 14 of PRD2010-19 is not accurate; the dermal developmental toxicity study *does* provide good comparative insight into the developmental consequences of exposure to acibenzolar via the dermal route, compared to oral ingestion. This study and the 28 day rat dermal toxicity study demonstrate that high concentrations of the chemical, under occlusive conditions, result in "no developmental or general toxicity following dermal absorption." These dermal toxicity studies aid in the risk assessment, support the safety of acibenzolar-S-methyl, and indicate that extra uncertainty factors are not needed for worker protection. The dermal exposure route is typically the most significant vector of exposure during product usage and post-application.

Response to Comment 3: The PMRA has stated, within the context of the hazard assessment, that "the dermal developmental toxicity study provides very limited comparative insight into the developmental consequences of dermal exposure to acibenzolar-S-methyl" because the amount that appears to have been absorbed in this study is far less than the orally administered dose levels that resulted in either the umbilical hernias or the brain morphometric changes (see Response to Comment 2). NOAELs were not established for these developmental toxicity effects. Thus, insight provided by the dermal developmental toxicity study is considered limited because the lowest dose capable of producing these developmental changes via the oral route was not achieved and the amount of acibenzolar-S-methyl actually absorbed in the dermal developmental toxicity study is not known. Additional uncertainty exists regarding the amount of acibenzolar-S-methyl that may ultimately be absorbed in humans because the physicochemical characteristics of the vehicle used in the rat dermal developmental toxicity study differ from those of Actigard 50WG, the end use product to which workers will be exposed. As noted, differences in vehicle composition make direct comparison of chemical absorption difficult (see Comment 2; difficulties introduced by the choice of vehicle). Further, since the brain morphometric effects noted in the DNT study were *not* assessed in the dermal developmental toxicity study it is not known whether the dose absorbed, or applied, led to comparable brain effects in this study. Finally, notwithstanding the low absorbed dose, the number of litters examined in this study was too few to reliably exclude the possibility that comparable rare developmental malformations might also occur with dermal exposure (see Response to Comment 1). Overall, the dermal developmental toxicity study offers very limited insight into establishing a clear NOAEL for developmental effects via the dermal route. As a result, the uncertainty factors proposed for worker protection are warranted.

The PMRA agrees that 28 days of exposure to acibenzolar-S-methyl via the dermal route resulted in a smaller spectrum of systemic toxicity effects, compared to the oral route. Nevertheless, these short-term studies had comparable NOAELs, regardless of the route of exposure. Like the dermal developmental toxicity study, the amount of acibenzolar-S-methyl that was actually absorbed during the 28 day dermal toxicity study is not known, and can only be inferred through information from the dermal metabolism study (see the response to comment 2). The PMRA agrees that these dermal toxicity studies do aid in the risk assessment and do suggest that exposure via the dermal route may result in fewer associated hazards, compared to the oral route. Nevertheless, the results of these studies are not sufficient to reduce or eliminate the uncertainty factors that have been recommended for worker protection.

Developmental Neurotoxicity

Comment 4: The PMRA stated on page 14 of PRD2010-19 that there was no evidence of maternal toxicity in the rat DNT study up to the highest dose tested, 4000 ppm. It is respectfully pointed out that in the similarly dosed two-generation rat reproduction study evidence of maternal toxicity was observed at 2000 and 4000 ppm. Given the smaller number of maternal parameters measured in the DNT study it is likely that maternal toxicity was present but not assessed.

Response to Comment 4: The PMRA agrees that the maternal effects in the reproductive study should be taken into consideration in the context of the DNT study. This is consistent with the PMRA's general approach of considering the entire toxicology database when assessing the hazard characteristics of a chemical. The maternal toxicity effects in the reproductive toxicity study were considered when assessing the absence of maternal toxicity in the DNT study. Consistent with Comment 2, regarding study comparisons, the PMRA also took into consideration that these two studies were conducted eight years apart by different laboratories on different strains of rat, provided by different animal suppliers, and which differed in their age, weight and the diet provided. These differences precluded a direct comparison between these studies.

Comment 5: Contrary to the PMRA's assessment of the morphometry results in the DNT study (page 14 of PRD2010-19), there was no dose response in the males for the changes in the dorsal cortex. In addition, the changes in the cerebellum were statistically significant at the mid and high dose only.

Response to Comment 5: The dose response curves for decreases in the thickness of the molecular layer of the cerebellum and the dorsal cortex both exhibited a response plateau. In the case of the cerebellum, the developmental change was beginning to plateau at the mid and high dose. For the dorsal cortex, a plateau was also evident at the lowest dose. Within a developmental context, this form of dose response may occur when there is a critical window of *time* in which a developmental process is susceptible to chemical toxicity. Under these circumstances, the magnitude of developmental change could be influenced by the administered dose *and* the duration of this fixed period of susceptibility. Alternatively, if the observed morphometric changes originate from the prenatal period, saturation of placental transfer may have contributed to the observed dose response characteristics. Regardless, the PMRA has no direct information regarding the effective dose to the pups during their development and no clear insight into the timing of the developmental susceptibility.

Although the changes in the cerebellum were statistically significant at the mid and high dose only, the change at the low dose was 78% and 55% of the change that occurred at the mid and high dose level, respectively. The change that occurred at the lowest dose was more than half of the change observed at the highest dose, despite a 40-fold dose increase. The degree of change at the low dose is considered biologically significant and underscores the steepness of the dose response curve below the low dose. Comparable dose response characteristics were also evident for dorsal cortex thickness, which exhibited statistically significant decreases at all dose levels tested. As noted in PRD2010-19, the steepness of these response curves below the lowest dose tested increased the PMRA's concern that very low doses of acibenzolar-S-methyl may substantially impact normal brain development.

The PMRA evaluated the brain morphometric data of control animals from thirteen historical DNT studies conducted by the same performing laboratory. These data indicate that brain morphometrics of the concurrent control group were generally not atypical and are not likely to have contributed to the dose response characteristics that were observed in the dorsal cortex and the cerebellum.

Comment 6: Most of the brain morphometric changes in the DNT study were within the range of the historical control data and there were no statistically significant changes in female animals. Giving consideration to all the findings, and considering the inherent variability in DNT studies, it is believed that the apparent changes in morphometry are not biologically or toxicologically significant and the developmental NOAEL in this study is the highest dose tested, 4000 ppm.

Response to Comment 6: Evaluation of the historical control data from the performing laboratory revealed non-random study-to-study variation in the brain morphometric measurements. For dorsal cortex thickness, this variation appears to be systematic and dependent on when the study was conducted. Comparable concerns were evident in the historical DNT behavioral data. This laboratory-driven variation broadens the historical control range in a manner that is unrelated to the inherent biological variation associated with normal brain structure development. It also underscores the central importance of the concurrent control group in assessing effects that appear to be related to treatment. Based on the historical control data provided, the PMRA established that the concurrent control data for male rats were not atypical and were not an underlying determinant of the morphometric changes observed. Contrary to commenter's belief, the PMRA considers the changes in treated male rats as being real, rather than apparent. The PMRA placed greater emphasis on the concurrent control group in its assessment, which is consistent with OECD guidance on mammalian reproductive toxicity testing (Number 43, ENV/JM/MOMO2008-16).

Although statistically significant changes were not detected among females, morphometric analyses in females were limited to the control and the high dose only. In addition, as indicated in PRD2010-19, the historical control data suggest that some masking of effects may have occurred, with instances of atypical control values also noted for some of the morphometric measurements.

Comment 7: In the first paragraph on page 15 of PRD2010-19, the PMRA's interpretation of the brain morphometric data is restated. Given the lack of dose response, only four statistically significant findings among twenty measurements of brain morphometry in male animals, the lack of concordant findings, and the United States Environmental Protection Agency's conclusion that, "The biological significance of the reduction in the dorsal cortex, which is not dose-dependent, is unclear", the changes in brain morphometry in the male animals do not appear to be "clear and consistent", as stated by PMRA.

Response to Comment 7: The PMRA stated that "In male pups, clear and consistent morphometric changes occurred down to the lowest dose tested…." The acceptability and limitations of the dose response were discussed in Response to Comment 5. The PMRA found spatial, temporal and developmental concordance as well as internal cohesion in the brain morphometric findings because: i) two independently measured regions of the dorsal cortex exhibited the same magnitude and direction of change, ii) two independently measured regions

of the cerebellum exhibited the same magnitude and direction of change, iii) two different brain regions exhibited the same type of gross morphological change, iv) both of the brain regions that were affected are late developing regions of the brain, v) there was evidence that the granular layer was similarly affected, vi) there was evidence that comparable, although not statistically significant, changes occurred in female pups. The regionally limited developmental effects of acibenzolar-S-methyl suggest that its mechanism of toxicity is specific rather than general, a possibility that is consistent with its toxic effects occurring to the lowest dose tested.

The PMRA concurs with the United States Environmental Protection Agency's view that the biological significance of a reduction in the dorsal cortex is unclear. However, based on current knowledge of the developmental timing of different regions and structures in the rat brain, aspects of the development of the dorsal cortex are known to be temporally concordant with those of the cerebellum. It is also known that an underlying biological basis for this developmental concordance is the presence of late-developing populations of granular cells in these two brain regions. Thus, the apparent temporal concordance of acibenzolar-S-methyl's effects in these two brain regions has a probable biological basis. Without further detailed knowledge of the timing and nature of the changes in both of the affected regions of the brain, the PMRA cannot rule out the possibility that these toxic effects are developmentally analogous.

Comment 8: The first two paragraphs on page 15 of PRD2010-19 include speculation which should be clearly indicated as such. There is no evidence of hypoplasia in any brain cells. Further, PMRA's comment that task stringency in this particular study was not sufficiently difficult is based on PMRA's extrapolation from the literature and not the data itself.

Response to Comment 8: Brain morphometric changes observed in the DNT study were clearly indicated in PRD2010-19. The PMRA was careful to distinguish between these study findings and the discussion on their potential underlying biological significance. The limits of the biological understanding of the observed brain developmental malformations were also clearly acknowledged. The PMRA agrees that the submitted DNT study contains no direct evidence of hypoplasia in any brain cells. However, the histological procedures used in the DNT study are not specifically intended to detect hypoplasia. Also, decreases in the thickness of these brain regions could have other underlying causes such as decreased cell size, increased cell death, altered cell migration, and in the case of the molecular layer decreased dendrite arborization. None of these possibilities is directly detectable with the histological procedures used in the DNT study.

The behavioral task conducted for learning and memory in the DNT study was acceptable and complies with the guidelines for this type of study. Prior to the conduct of the submitted DNT study, it would not have been possible for Syngenta or the PMRA to know which behavioral task, or level of stringency, might be the most appropriate to investigate. Nevertheless, in light of our current understanding of brain development, the detected brain morphometric changes should be used to inform the design of any subsequent studies, should they be conducted. Similarly, the brain morphometric changes that occurred should be considered retrospectively, to assess the probable suitability and sensitivity of behavioral tasks that were actually conducted in the DNT study.

Comment 9: On Page 17 of PRD2010-19, the PMRA states that "The DNT brain developmental effects and the umbilical hernias occurred at essentially the same low dose level". It is not believed this is an accurate reflection of the data. In the DNT study the exposure was via the diet and in the developmental toxicity study the exposure was by oral gavage, using aqueous carboxymethylcellulose as the vehicle. The kinetics of acibenzolar-S-methyl was therefore very different in the two studies. Clarification from the PMRA as to the reasoning behind this statement is appreciated.

Response to Comment 9: The PMRA agrees that a chemical's absorption kinetics are influenced by the properties of the vehicle used to deliver the dose. However, the toxicology database for acibenzolar-S-methyl does not include a comparative analysis of the absorption kinetics in rats that were exposed via either their diet or using aqueous carboxymethylcellulose as the vehicles. Further, the vehicle used to characterize the absorption kinetics in the oral metabolism study was a mixture of N-methylpyrolidone and polyethylene glycol, which differs from the vehicles used in these developmental toxicity studies. Bearing these limitations in mind, the "dose", as referred to in PRD2010-19, necessarily refers to the average amount of chemical provided daily, per kilogram of body weight, via the oral route of exposure. The PMRA has not been provided with specific toxicokinetic information that would aid in determining the biologically effective dose for the different vehicles used in the oral developmental toxicity studies.

Comment 10: A review of all the findings in the DNT study indicates an absence of developmental neurotoxicity. As a result the extra 3-fold uncertainty factor may be removed. The United States Environmental Protection Agency has assigned a NOAEL to the rat DNT study of 8.2 mg/kg. In addition the appropriate definition of a NOAEL means that the PCPA factor, if applied, may be restricted to females ages 13 to 49.

Response to Comment 10: The presence of developmental neurotoxicity effects in rats following exposure to acibenzolar-S-methyl is a consistent finding of the PMRA and the United States Environmental Protection Agency. The PRD2010-19, and Responses to Comments 4 though 8, offer insight into why the PMRA assessment of the DNT study differs somewhat from that of the United States Environmental Protection Agency. A 3-fold uncertainty factor was applied due the absence of a NOAEL; the dose response curve is discussed in PRD2010-19 and in Response to Comment 5. A PCPA factor of 10-fold was applied for the reasons outlined in PRD2010-19 under Section 3.1.1. Concerns relating to the application of a PCPA factor are independent of those relating to the application of a 3-fold uncertainty factor for the lack of a NOAEL. The PCPA concerns relating to developmental neurotoxicity apply to all populations because there was insufficient information in the DNT study to establish whether the brain morphometric changes resulted from exposure to acibenzolar-S-methyl during the prenatal period, the postnatal period, or both. This exposure issue is further complicated by differences in the developmental timing of the affected brain regions in rats and humans.