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Post-harvest and Storage Solutions

Project history: Diphenylamine

DPA is a plant growth regulator used indoors post-harvest on apples and pears to control storage scald (common scald, superficial scald) prior to entering storage. DPA is an anti-oxidant, which prevents oxidation of the naturally occurring terpenes, thereby controlling development of storage scald.

Diphenylamine (DPA) is a List 3B substance. The DPA Task Force submitted **a 91/414/EEC dossier on DPA** to the PCS in Ireland (Rapporteur Member State) in November 2004. Please note that in the original submission two forms of application were supported, dipping and drenching. However, during the review of the DPA data package, the PCS requested that further 3 residue trials must be submitted to support a dipping application. The DPA Task Force decided to no longer support a dipping application and therefore this requirement was no longer valid. Drenching was the only supported application method.

The original formulation had very high levels of nonylphenols (>20%). The new EU permissible level is <1%. It was agreed, with the Task Force, to compromise with the November 2004 EU submission and submit the formulation with nonylphenols, but to advise the RMS of the formulation change to non-NP formulations. Once the new formulation data was available it was included as part of **an updated Annex III submission** which was presented to PCS at the time of the completeness check meeting (17/02/2005). The final report, and summaries, for an apple metabolism study was also presented at this time.

Following the completeness check meeting, certain areas were highlighted by the PCS as requiring further information. This information was presented to the PCS.

An Addendum to the Dossier was submitted in October 2006 to the PCS in response to queries received as part of the evaluation of the DPA Dossier. Individual queries raised were also responded to as part of the Addendum.

A final version of the dossier which includes the updates of the Annex III, implemented comments post completeness check and the Dossier Addendum was circulated to the European Food Safety Authorities (EFSA) and all the MS's.

A **Draft Assessment Report (DAR)** was prepared and sent to Rivendell in May 2007 for editorial comments and minor feedback was provided to PCS at this time. A final version of the DAR from PCS was received in June 2007. The DAR was sent to the EFSA at this time. As part of the DAR, the ***RMS recommended Annex I inclusion*** for DPA pending further clarification on some areas of the submission. An updated summary dossier, along with CADDY versions, was circulated by Rivendell to the EFSA and the other MSs in August 2007. The DPA DAR was consequently sent by EFSA to all MS's and Rivendell (acting as representative for the DPA Taskforce) for comment. The deadline for submitting all comments on the DAR, to the EFSA, was 7th December 2007.

On the 26th of November 2007, it was decided with the Task Force that DPA would continue to Peer Review. As a result the DAR was reviewed and the commenting table was sent to the PCS. PCS then had to respond to these comments and decide weather or not the issues raised could now be closed out on. This version of the reporting table was sent to the EFSA on the 8th of February 2008. EFSA then had to decide what issues were going to be discussed at Peer Review. PCS had recommended that the procedure to be followed now be a focused Peer Review on the small number of open points indicated in the reporting table. PCS also expressed the view that there were no issues for referral to the PPR Panel.

The data gaps found at this stage are as follow:

- Information on starting material: a specification of DPA purchased was missing.
- The spectra of relevant impurities had to be provided.
- Evidence to demonstrate that when opened containers are stored, DPA remains stable had to be provided.
- The emulsion stability at the minimum in use concentration had to be provided.
- Method of analysis for products of animal origin had to be provided.
- An acute inhalation toxicity study had to be provided.
- The impurity profile of the batches used in the toxicological studies had to be provided.
- It had to be proved whether there is any probability for the formation of nitrosamines in metabolism.
- Issue with potential residues in food of animal origin was proposed.
- Clarification was required on the processing studies with regards to the residue levels in wet and dry pomace.
- Further data was required on the nature of the residues in apple processed commodities.

DPA was put on the 'yellow' route **even though PCS were recommending the 'green'** route. As it was yellow routed, the Task Force had to make a decision as to whether they were going to stay on the current registration or take the voluntary withdrawal option. A meeting between Rivendell and the PCS took place to discuss this in more detail and to focus in on areas of the dossier that may require further work. The main issues of concern were the potential presence of nitrosamines during processing and the unidentified metabolites in the metabolism study. There were also some other data gaps and open points that needed to be addressed, however it was felt that the above two points were the main reason for non-inclusion. Subsequently a ***non-inclusion decision*** was reached by the Commission on 30th November 2009 **(*Decision 2009/859/EC*).**

The DPA Task Force decided to address the open points raised by EFSA and to re-submit the dossier under the accelerated procedure according to Regulation (EC) No 33/2008. The re-submission deadline was established on the 30th May 2010. As part of this resubmission procedure, new studies needed to be generated to address some data gaps in the original dossier. So, the following studies were commissioned:

Study	**Laboratory**
Product Emulsion Stability	HLS
Spectra of impurities	Chemservice
Acute inhalation study on DPA	Covance
TLC method to demonstrate absence of nitrosamines	Charles River
Identification of Metabolites/Processing Study	Charles River

Emulsion stability and *Impurities spectra tests* were finished without any relevant issue. However, regarding the *acute inhalation study in rats* it was not possible to generate a stable aerosol of DPA using standard inhalation laboratory equipment. An expert statement was prepared to justify these results and it was considered acceptable by the Authorities.

Previous studies on the stability of apples, apple juice and wet and dry apple pomace as well as the effects of processing and household preparations on these materials when apples are treated post harvest were submitted in the original dossier. Results shown the DPA and its residues found in the samples were stable under freezing conditions. Nevertheless, Member States requested further information on DPA metabolism in order to clarify the potential formation of breakdown or reaction products of DPA in processed commodities, especially when referred to nitrosamine derived metabolites. In order to address all the information regarding DPA metabolism and identification of metabolites present in processed commodities the study "*Investigation into the Nature and Identity of Radiolabelled Components Present in Processed Apple Commodities Following Treatment with [^{14}C]-DPA and Storage for 12 and 36 Weeks*" was performed. This study should be performed over a time period of 40 weeks, but timelines were very tight and finally a study on 36 weeks was accepted by the Authorities. The mayor metabolite that was found in processing study was DPA. Furthermore, samples from a previous study (Inveresk 804015 (Gray, J.L. 2005)) were selected for mass spectral analysis in order to attempt to identify unknowns 1-3 that

were detected. The analysis of these samples showed a higher number of metabolites that those found in Inveresk 804015 study. Unfortunately, it was not possible to resolve chromatogram peaks and no reliable masses could be determined with most adequate and sensitive technique HPLC-MS. A chromatographic comparison (HPLC and 2D TLC) of N-nitrosodiphenylamine and DPA was attempted in order to demonstrate that any of the unknown metabolites present in the samples are nitrosamine-derived compounds. It was confirmed by two techniques that no nitrosamine functional groups were present in the samples.

The Resubmission Dossier summarising the new data was sent to the PCS on the 26th of May 2010 and the finalised processing study was submitted to the PCS as additional information on the 18th of November 2010.

An Additional Report to the DAR was prepared by the PCS and released in November 2010. Following the review of the Additional Report by EFSA and the Member States, a reporting table with their comments was received in February 2011. Some clarifications had to be provided as well as two new studies were required in order to clearly demonstrate the absence of nitrosamine formation in the processed commodities. All the open points and questions from Member States and EFSA were answered in the Reporting table Document for DPA (rev. 0 (10-02-2011)) and the Task Force showed their willingness to perform these new studies. These two new studies were the simulated *Hydrolysis study* and the *Validation of the analytical method used for the determination of nitrosamines*. The corresponding contracts have been already signed with Charles River and in the coming weeks, protocols for these new studies will be prepared and need to be reviewed.

The data gaps found at this stage are as follow:

- Information on starting material: a specification of DPA purchased was missing.
- A validated analytical method should be provided in relation to the method for the determination of nitrosamines in treated apple samples.
- A simulated hydrolysis studies was required.

On the other hand, on the 4th of May 2011, the DPA Task Force received a communication from the PCS informing them about the recent draft opinion on DPA which EFSA revised in the framework of the Regulation 396/2005 (MRL, Maximum Residue Level establishment). In principle, the final version of the EFSA opinion will be published before October 2011, however it is not clear whether this opinion will consider the new data submitted as part of the resubmission dossier (as the draft does not include any reference to the new data submitted). This fact concerns both the Task Force and the PCS. The PCS agreed to apply for an extension to the time of the Annex I resubmission is completed. On the other hand, as part of this communication, the PCS established a deadline on the 22nd of May 2011 to receive any application for import tolerances that may be requested for DPA. Therefore, an **application for setting the import tolerances/maximum residue levels for DPA** in apples and pears was submitted on the 17th of May 2011. This information was accepted by the PCS and was submitted to EFSA for its evaluation. Taking advantage of this submission and after having the approval from the PCS, the Task Force agreed to commission the two studies (validation method for the determination of nitrosamines and hydrolysis of DPA) that are needed to satisfy the Member States and EFSA's requirements and to clarify whether the formation of nitrosamines could be expected or not in processed commodities. These two studies are to be started in August and September 2011. On the last meeting between the PCS, the EFSA and the Commission that took place in Brussels on the 18th of May 2011, the PCS informed both the Commission and the EFSA about the Task Force's intention which involves to take advantage of the import tolerance application in order to submit two new studies that will help both to set the import tolerance and to address the data gaps found in the resubmission dossier. Although these applications (Setting import tolerances and Resubmission dossier) follow two different procedures, the PCS is trying to use the new data to support both applications. In order to do that, the Commission needs to agree to provide the DPA with an extension of time. In their discussions at the meeting, EFSA delegate considered logical an extension to the time of the Annex I resubmission. However, the Commission cannot agree anything with this regard until they review the EFSA MRL opinion.

Therefore, after the last communication with the PCS, they informed Rivendell that the following procedure will take place: the EFSA opinion is published, then the Commission will bring DPA to the Standing Committee (October at the earliest) and it is at that point when the Commission will get the EFSA MRL opinion. At this point in time, the PCS will look officially for an extension to the time of the Annex I resubmission is completed, although the PCS has already informed the Commission, outlining the background and the importance to defer a decision in the light of potential impacts on trade when the EFSA opinion is published.

Regarding the two new studies that will be commissioned, Charles River needs to evaluate the suitability (purity) of the stored radiolabeled samples. This preliminary analysis will be added to one of the protocols and once the protocol is signed and they have performed this analysis, they will let us know how pure the compound is and what action is required. In addition, according to the PCS recommendation and after discussing with Charles River, the Mass Spectroscopy (MS) detection will be required for the validation. The level of the metabolites (possible nitrosamines) present in the apple samples from the processing study mean that LC-DAD or LC-UV would not be appropriate. The aim of the validation study is also to determine the lower limit of detection for nitrosamines and therefore the lab would always perform this analysis by MS. The system that Charles River will be using for the validation is an API5000 MS instrument in Multiple Reaction Monitoring (MRM) mode. This is the most sensitive instrument that they have on-site and therefore it should give them the best results for the validation.

Please find in Annex I the chronological order of events in relation to the active substance DPA and in Annex II the forthcoming events.

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ANNEX I

Chronological Order of Events in relation to the active substance DPA:

Event	Date
Diphenylamine Dossier submitted to RMS	November 2004
Completeness Check Meeting	February 2005
Addendum to the Dossier submitted to the RMS	October 2006
Rivendell Received an editorial DAR for minor comments	May 2007
Final DAR received	June 2007
Circulation of Dossier (containing addendum) to each Member State	August 2007
DPA continued to Peer Review	November 2007
Deadline for submitting all comments on the DAR, to the EFSA	December 2007
EFSA comments on DAR on the apple metabolism study and processing in the format of a reporting table.	January 2008
Reporting table sent to EFSA	February 2008
Evaluation table	June 2008
Data Requirements from EFSA received	August 2008
Peer Review Report on DPA	October 2008
Non-inclusion decision on DPA (Commission)	November 2009
Deadline for submitting the re-submission dossier to the PCS	May 2010
Finalised processing study submitted to the PCS	November 2010
Additional Report to the DAR was received from the PCS	November 2010
Reporting table with the EFSA and MS's comments received	February 2011
Reporting table sent to EFSA	February 2011
DPA classification proposal received from the PCS	March 2011
Comments on the DPA classification proposal sent to the PCS	March 2011
EFSA's Draft opinion on DPA resubmission received	May 2011
Deadline for submitting MRL/import tolerance applications	May 2011
Import tolerance application submitted to the PCS	May 2011

ANNEX II

Forthcoming Events in relation to the active substance DPA:

Event	Date
Protocols for the hydrolysis study and the validation of the analytical method will be completed	July-August 2011
Final report on the Validation of the analytical method	September 2011
Final report on the Hydrolysis study	October 2011
EFSA's opinion on DPA MRL wil be published	Nov/Dec 2011

ANNEX III

European Acronyms

RMS; Reporting Member State (for this project Ireland was appointed)

RIVENDELL; Task Force consultant and agent

DAR; Draft Assessment Report

PCS; Product Chemistry Service (for this project, Ireland Department of Agriculture)

MS; Member State.