



# ***In silico* modelling of microbial and human metabolism: a case study with the fungicide carbendazim**

**Aleksandra Mostrag-Szlichtyng and Andrew P. Worth**

EUR 24523 EN - 2010

The mission of the JRC-IHCP is to protect the interests and health of the consumer in the framework of EU legislation on chemicals, food, and consumer products by providing scientific and technical support including risk-benefit assessment and analysis of traceability.

European Commission  
Joint Research Centre  
Institute for Health and Consumer Protection

**Contact information**

Address: Via E. Fermi 2749, 21027 Ispra (VA), Italy  
E-mail: [andrew.worth@ec.europa.eu](mailto:andrew.worth@ec.europa.eu)  
Tel.: +39 0332 789566  
Fax: +39 0332 786717

<http://ihcp.jrc.ec.europa.eu/>  
<http://www.jrc.ec.europa.eu/>

**Legal Notice**

Neither the European Commission nor any person acting on behalf of the Commission is responsible for the use which might be made of this publication.

***Europe Direct is a service to help you find answers  
to your questions about the European Union***

**Freephone number (\*):**

**00 800 6 7 8 9 10 11**

(\*) Certain mobile telephone operators do not allow access to 00 800 numbers or these calls may be billed.

A great deal of additional information on the European Union is available on the Internet. It can be accessed through the Europa server <http://europa.eu/>

JRC60264

EUR 24523 EN  
ISBN **978-92-79-16744-7**  
ISSN 1018-5593  
doi:**10.2788/98567**

Luxembourg: Publications Office of the European Union

© European Union, 2010

Reproduction is authorised provided the source is acknowledged

*Printed in Italy*

## ABSTRACT

A major source of uncertainty when assessing the human health and environmental risks of chemicals is the paucity of experimental information on the metabolic and (bio)degradation pathways of parent compounds and the toxicological properties of their metabolites and (bio)degradation products. Taking into account animal welfare and cost-effectiveness considerations, the only practical means of obtaining the information needed to reduce this uncertainty, is to use alternative (non-animal) methods, such as *in vitro* tests and *in silico* models.

In this report, we explore the usefulness of *in silico* metabolic simulation tools (expert systems) as a means of supporting the regulatory assessment of chemicals. In particular, we investigate the use of selected *in silico* tools to: (i) simulate microbial and mammalian metabolic pathways; (ii) identify potential metabolites resulting from biotransformation; and (iii) gain insights into the mechanistic rationale of simulated metabolic reactions and the likelihood of their occurrence. For illustrative purposes, the microbial and mammalian biotransformation pathways of a case study compound, the fungicide carbendazim, were generated by using the CRAFT Explorer 1.0 (Molecular Networks GmbH) and Meteor 12.0.0 (Lhasa Ltd.) software tools. Additionally, the set of potential metabolites resulting from microbial and mammalian metabolism was predicted with the OECD QSAR Application Toolbox 2.0 (beta version).

Comparison of the *in silico* predictions with existing experimental data on carbendazim metabolism showed the potential usefulness of using software tools for metabolite prediction. However, the results are strongly dependent on the software constraints specified by the user, and require careful interpretation, taking into account the needs of the exercise and the availability of existing information. Further efforts are needed to develop guidance on the use of *in silico* metabolic simulation tools for the purposes of regulatory risk assessments.

## LIST OF ABBREVIATIONS

2-AB	2-Aminobenzimidazole
5,6-DHCB	Methyl (5,6-dihydroxy-1H-benzimidazol-2-yl)carbamate
5,6-DHCB-G	Glucuronic acid conjugate of methyl (5,6-dihydroxy-1H-benzimidazol-2-yl)carbamate
5,6-DHCB-S	Sulphuric acid conjugate of Methyl (5,6-dihydroxy-1H-benzimidazol-2-yl)carbamate
5,6-DHHBC-G	S-[5,6-dihydro-5-hydroxy-2-(methoxycarbonylamino)-1H-benzimidazol-6-yl]glutathione
5,6-HOBC N-oxide	Methyl (5-hydroxy-6-oxo-6H-benzimidazol-2-yl)carbamate N1-oxide
5,6-HOBC N-oxide-G	Glucuronide of Methyl (5-hydroxy-6-oxo-6H-benzimidazol-2-yl)carbamate N1-oxide
5-HCB	Methyl 5-hydroxy-1H-benzimidazol-2-ylcarbamate
5-HCB-G	Glucuronic acid conjugate of Methyl 5-hydroxy-1H-benzimidazol-2-ylcarbamate
5-HCB-S	Sulphuric acid conjugate of Methyl 5-hydroxy-1H-benzimidazol-2-ylcarbamate
AGES	Austrian Agency for Health and Food Safety
Bt	Biotransformation
CAS No	Chemical Abstracts Service Registry Number
CRAFT	Chemical Reactivity and Fate Tool
CYP450	Cytochrome P450
EC	European Commission
EEC	European Economic Community
EFSA	European Food Safety Authority
EU	European Union
FAO	Food and Agriculture Organisation
IUPAC	International Union of Pure and Applied Chemistry
JMPR	Joint FAO/WHO Meeting on Pesticide Residues
JRC	Joint Research Centre (of the European Commission)
Log P	Octanol/Water partition coefficient
M	Meteor 12.0.0 Metabolite
OECD	Organisation for Economic Cooperation and Development
P	CRAFT Explorer 1.0 (Bio)Degradation Product
PPP	Plant Protection Product
Q	Meteor 12.0.0 Query Compound
QSAR	Quantitative Structure-Activity Relationship
SA	Structural Alert
SAR	Structure-Activity Relationship
SMILES	Simplified Molecular Input Line Entry Specification
TIMES	Tissue Metabolism Simulator
TTC	Threshold of Toxicological Concern
UGT	UDP-glucuronosyltransferase
UM-BBD	University of Minnesota Biocatalysis and Biodegradation Database
WHO	World Health Organisation

# CONTENTS

<b>1. Introduction .....</b>	<b>1</b>
<b>2. Characteristics of the fungicide carbendazim .....</b>	<b>3</b>
<b>3. Simulating microbial metabolism of carbendazim with CRAFT .....</b>	<b>6</b>
3.1. CRAFT Explorer methodology and model details .....	6
3.2. Carbendazim biodegradation tree .....	7
<b>4. Simulating mammalian metabolism of carbendazim with Meteor.....</b>	<b>13</b>
4.1. Meteor methodology and model details .....	13
4.2. Carbendazim metabolic tree .....	13
<b>5. Predicting carbendazim metabolites with the QSAR Application Toolbox.....</b>	<b>20</b>
<b>6. Discussion and conclusions .....</b>	<b>23</b>
6.1. Biodegradation of carbendazim.....	23
6.2. Mammalian metabolism of carbendazim .....	23
6.3. Parallels between microbial biodegradation and mammalian metabolism .....	24
6.4. General conclusions and recommendations .....	26
<b>7. Disclaimer and acknowledgements .....</b>	<b>29</b>
<b>8. References .....</b>	<b>29</b>



## 1. Introduction

One of the most important ways of protecting plants and plant products against harmful organisms and of improving agricultural production is the use of plant protection products (agricultural pesticides). However, a possible consequence of their use may be the presence of pesticide residues in the treated products.

The broad application of plant protection products on food commodities results in the increased exposure of consumers, not only to the residues of the active components, but also to a wide range of their metabolites, degradation and reaction products. As the total burden of all toxicologically significant substances should be taken into account for human health and environmental risk assessments, data on metabolism in mammals, birds, livestock, plants, needed along with data on environmental fate (including microbial degradation in soil or water/sediment). In combination with toxicity data, this information provides a broad experimental basis for the risk assessment.

Metabolism is one of the main factors influencing the fate and toxicity of chemicals. For instance, microbial metabolism (e.g. in soil or water) affects the overall environmental persistence of chemicals and the duration of their presence and potential activity in particular environmental compartments. On the other hand, biotransformations inside organisms (e.g. mammals), which generally convert xenobiotics into more polar and more easily excreted forms, regulate the internal exposure conditions. Metabolites that are not toxicologically significant can be excluded from further investigations. In some cases, however, the metabolites represent equal or higher toxicity than the parent compounds and these need to be considered to ensure that the risk assessment provides adequate protection of human health and the environment

A major difficulty stems from the fact that only the toxicological properties of the active substance are normally directly investigated through the range of toxicological studies required according to legislation such as *Directive 91/414/EEC* (EC, 1991) in the European Union. However, according to the OECD guidance document on the definition of residue (OECD, 2009), all metabolites the consumer is exposed to in plant and animal commodities should be considered in terms of their relevance for risk assessment. In the case of metabolites present in food commodities that are also significant metabolites in rodent studies, their toxicity is considered to be implicitly assessed in the rodent study. Data gaps arise for metabolites that are not considered to be significantly present in rodents. Since further toxicological studies are restricted as far as possible to minimise the use of animals in toxicological testing, alternative (non-animal) methods need to be developed further and used to optimise the evaluation the toxicological profile of metabolites and degradates. For example, *in silico* methods that simulate metabolic and degradation reactions, and which predict ranges of potential (theoretically possible) metabolites/products, could be used to guide further toxicological assessments. The results obtained with these simulation methods could be applied in combination with Threshold of Toxicological Concern (TTC) considerations (EFSA, 2010a), existing knowledge on the impact of metabolic and degradation processes on pesticide residue toxicity (EFSA, 2010b), as well as *in silico* toxicity predictions (EFSA, 2010c).

Several *in silico* approaches for modelling metabolism can be distinguished, e.g. statistical models (Okey & Stensel, 1996; Boethling, 1986), group contribution models (Tunkel et al., 2000; Boethling et al., 1994) or models based on enzyme activity and substrate specificity (Vermulen, 2003). Models for predicting microbial metabolism (biodegradation) and

mammalian metabolism are reviewed in depth elsewhere (Pavan & Worth, 2008; Mostrag-Szlichtyng & Worth, 2010).

In this report, we explore the usefulness of *in silico* metabolic simulation tools (expert systems) as a means of supporting the regulatory assessment of chemicals. In particular, we investigate the use of selected *in silico* tools to: (i) simulate microbial and mammalian metabolic pathways; (ii) identify potential (theoretically possible) metabolites resulting from biotransformation; and (iii) gain insights into the mechanistic rationale of simulated metabolic reactions and the likelihood of their occurrence.

For illustrative purposes, the microbial and mammalian biotransformation of a case study compound, the fungicide carbendazim, was performed by using the CRAFT Explorer 1.0 (Molecular Networks GmbH, Germany) and Meteor 12.0.0 (Lhasa Ltd., UK) software tools. Additionally, the set of potential microbial and mammalian metabolites was predicted with the OECD QSAR Application Toolbox 2.0 (beta version), which implements the hybrid (statistical and rule-based) methodology to predict metabolism (Mekenyan et al., 2004). The results of the simulations were compared with existing experimental data on the mammalian metabolism of carbendazim in order to evaluate the applicability of the *in silico* tools.



## 2. Characteristics of the fungicide carbendazim

Carbendazim was first included in Annex I of the Plant Protection Products Directive (*Directive 91/414/EEC*; EC, 1991) on 1 January 2007 by Commission Directive 2006/135/EC, as amended by Commission Directive 2009/152/EC. Annex I is the list of active substances that are authorised for use in plant protection products within the European Union.

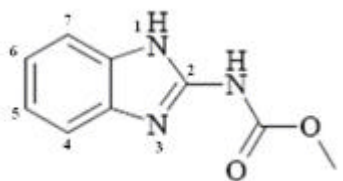
Carbendazim was recently re-evaluated by the European Food Safety Authority (EFSA), following an industry request to the European Commission for renewal of the Annex I inclusion (which is due to expire on 31 December 2010). The conclusions of the EFSA peer review (EFSA, 2010d) identify a number of data gaps, including the route of aerobic biodegradation in soil. Thus, the possibility to model metabolite generation by aerobic biodegradation in soil (or water/sediment) could be useful in the assessment of fate.

To explore the possibility of filling this data gap by using metabolic simulation tools, the CRAFT Explorer 1.0 software tool was used to simulate the biodegradation pathways of carbendazim and to generate a range of potential metabolites.

Since the mammalian metabolism of carbendazim has been investigated extensively, existing experimental data could be used to assess the applicability of metabolism simulations performed in this study (i.e. Meteor 12.0.0 and OECD QSAR Application Toolbox 2.0) in this particular case.

Carbendazim is the common name for methyl benzimidazol-2-ylcarbamate or 2-(methoxycarbonylamino)-benzimidazole (IUPAC). The main information concerning its chemical identification as well as physical and chemical properties is provided in Table 1.

**Table 1.** Chemical identity and physicochemical properties of carbendazim (EFSA, 2010d)

Chemical name (IUPAC)	Methyl benzimidazol-2-ylcarbamate (MBC); 2-(Methoxycarbonylamino)-benzimidazole	Melting point	302 – 307 °C (under decomposition)
Chemical name (CA)	Methyl 1H-benzimidazol-2-ylcarbamate	Boiling point	Not applicable
CAS No	10605-21-7	Appearance	Pure: almost colourless crystalline solid, odourless; Technical: light yellow to light grey crystalline powder, odourless
SMILES	<chem>c12c(cccc1)N=C(NC(=O)OC)N2</chem>	Vapour pressure	$9 \times 10^{-5}$ Pa (20 °C); $1.5 \times 10^{-4}$ Pa (25 °C)
Molecular formula	$C_9H_9N_3O_2$	Henry's law constant	$3.6 \times 10^{-3}$ Pa m <sup>3</sup> /mol (24 °C)
Molecular mass	191.21 g/mol	Water Solubility	pH 4: 29 mg/L; pH 7: 8 mg/L; pH 8: 7 mg/L
Structural formula		Partition coefficient	pH 5: $\log P_{O/W} = 1.4$ (25 °C); pH 7-9: $\log P_{O/W} = 1.5$ (25 °C)
		Dissociation constant	pKa = 4.2

Recently, EFSA identified data gaps for the following properties of carbendazim: UV spectra, solubility in organic solvents, dissociation constant, flammability, auto-flammability, explosive properties, oxidising properties and surface tension, soil photolysis, route of aerobic degradation in soil (EFSA, 2010d).

Carbendazim is a wide-spectrum systemic fungicide, belonging to the chemical class of highly selective benzimidazoles. It is used as plants (e.g. cereals, sugar beet, fodder beet, oilseed rape, maize, fruit and vegetables) disease controlling agent, effective against many ascomycetes, basidiomycetes and deuteromycetes. It can be applied to prevent either crops (in pre- and postharvest treatment) or stored postharvest food (EFSA, 2010b; Magnucka et al., 2007).

The mode of action of benzimidazoles relies on intensive binding to fungal microtubules (tubulin  $\beta$ -subunit) and disrupting various cellular processes (e.g. formation of the mitotic spindle, nuclear division, cytoskeleton formation), resulting in the cells death. The selective toxicity of benzimidazole fungicides and their relative potency towards different organism varies widely and is highly dependent on the structure of both the chemical and the respective tubulin, as well as on the fact that benzimidazoles show enhanced affinity to fungal microtubules. Nevertheless, their presence in other eukaryotes can also cause adverse effects on cellular functions. Benzimidazoles can initiate chromosomal aberrations, changes in chloroplasts (and, thus, photosynthesis), in germination and growth of seedlings. They may also impair other physiological processes and modify plant cells metabolism as well as contribute to morphological changes in plants (EFSA, 2010b; Magnucka et al., 2007; Kiso et al., 2004; Bilinski et al., 1984). The fact that benzimidazole fungicides can be intensively absorbed on soil organic matter and imbibed by non-target plants leads to concerns about their adverse effects on treated plant crops (Goralczyk et al., 2005; Berglof et al., 2002).

As far as the mammalian toxicity of benzimidazole fungicides, their metabolites and metabolic pathways is concerned, numerous studies were conducted. Since carbendazim is not only a parent “active” constituent of technical fungicide formulations, but also the metabolite of two other benzimidazole fungicides, namely benomyl and thiophanate-methyl, the data can be analyzed in detail and compared in these cases (EFSA, 2010b).

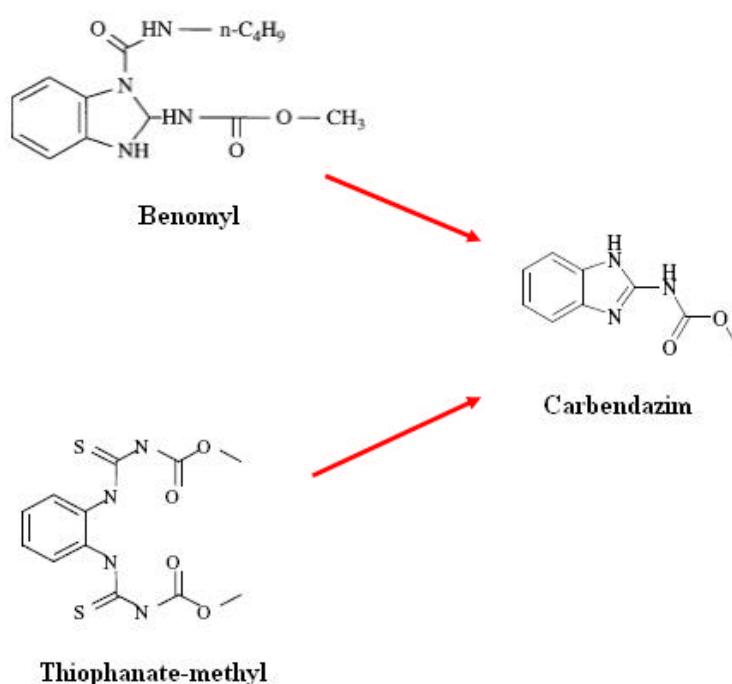
The studies concerning mammalian toxicity of carbendazim showed that it is not acutely toxic and is not a skin/eye irritant, but is a skin sensitizer. Short-term toxicity studies performed on rats and dogs resulted in the increased liver weight, reduced testes weight, azoospermia, decreased body weight and lower food consumption. Carbendazim caused numerical chromosome aberrations (*in vitro* and *in vivo*) as a result of the interference with mitotic spindle proteins, but did not cause gene mutations or structural chromosomal aberrations. In long-term toxicity studies in rats, mice and dogs, increased liver weight was observed. Reproductive toxicity studies in rats showed that carbendazim produces infertility in males, decreased sperm counts, testicular atrophy and absence of spermatogenesis. Studies on developmental toxicity by oral gavage in rats and rabbits demonstrated that carbendazim is a developmental toxicant and a teratogen (EFSA, 2010b).

Based on the effects described above, classification and labelling with R43 (may cause sensitisation by skin contact) in addition to the current classification and labelling as Mutagen Cat. 2 (R46) and Repr. Cat. 2 (R60-61) was proposed for carbendazim (EFSA, 2010b).

As far as toxicity of carbendazim metabolites is concerned, one acute and one short term toxicity study with the metabolite 2-AB (2-aminobenzimidazole) were conducted (EFSA, 2010b). 2-AB is formed by the cleavage of acetic acid of carbendazim and showed higher acute and equal short term toxicity compared to parent (EFSA, 2010b).

As mentioned before, carbendazim can be formed as a principal metabolite of other benzimidazole fungicides. It can originate from a fungicide benomyl (IUPAC name: methyl 1-(butylcarbamoyl)benzimidazol-2-ylcarbamate) by the cleavage of the butylcarbamoyl side chain of parent. It can be also formed from thiophanate-methyl by the cleavage of the thioallophanate chain, cleavage of the sulphur and cyclisation to form the imidazole moiety (Figure 1).

When the toxicity of carbendazim (as metabolite) is compared with the toxicity of the relevant precursors, the following conclusions may be drawn: (i) compared to Benomyl, carbendazim has equal acute toxicity, higher short and long term toxicity and equal reproductive and developmental toxicity; (ii) compared to thiophanate-methyl, carbendazim has equal acute and long term toxicity and higher short term, reproductive and developmental toxicity (EFSA, 2010b).



**Figure 1.** Carbendazim as a principal metabolite of benomyl and thiophanate-methyl

As far as environmental fate and behaviour of carbendazim are concerned, EFSA identified lacking information on its route of aerobic degradation in soil and transformation products formed as a result of microbial activity in soil. As a consequence, the environmental exposure assessment for potential soil metabolites could not be finalised for the representative uses at EU level (EFSA, 2010d).

### 3. Simulating microbial metabolism of carbendazim with CRAFT

#### 3.1. CRAFT Explorer methodology and model details

For the purpose of filling the gap in scientific data (EFSA, 2010d), the simulation of carbendazim biodegradation pathway was performed with the CRAFT (Chemical Reactivity and Fate Tool) Explorer v. 1.0 software, developed by Molecular Networks GmbH (<http://www.molecular-networks.com/>). CRAFT Explorer uses a knowledge base of different environmental reaction types derived from the University of Minnesota Biocatalysis and Biodegradation Database (UM-BBD, <http://umbbd.msi.umn.edu/>). The details of methodology underlying CRAFT Explorer predictions are described in the CRAFT Explorer user manual.

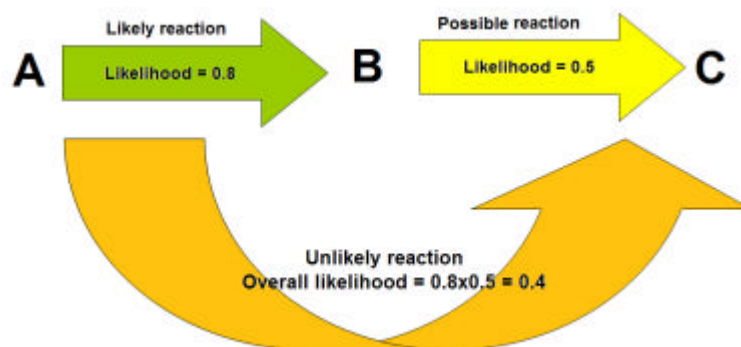
Briefly, the evaluation of the chemical reactivity and fate of carbendazim was based on the simulated degradation pathway, including a set of degradation reactions that carbendazim undergoes and the set of imaginable products. CRAFT Explorer was used to generate all the conceivable reactions starting from the investigated molecule (i.e. carbendazim) up to a specified limit. The final reaction generation required preselection of three variables: (a) a likelihood model applicable to an evaluation; (b) stop criteria defining when the program should cancel any further degradation and (c) a likelihood threshold.

The CRAFT Explorer **likelihood models** consist of several reaction rules, reaction types and likelihood categories assigned to each single rule. *Reaction type* is a general definition of a chemical transformation provided in the form of a reaction and annotated with the necessary substructure features and transformation definitions. *Reaction rule* implements certain reaction type and connects the general definition with the specific reaction conditions and likelihood estimations. From two likelihood models available in the software, namely the UM-BBD likelihood model and the ester hydrolysis sample model, the first one was selected. The UM-BBD likelihood model implements the biotransformation rules defined by Ellis et al. (2006) and available at the UM-BBD web site (<http://umbbd.msi.umn.edu/>). The reaction rules evaluated in the scope of UM-BBD likelihood model were considered for biodegradation under aerobic conditions, in soil (moderate moisture) or water, at neutral pH, in 25°C, with no competing or toxic other compounds (<http://umbbd.msi.umn.edu/predict/rulepriority.html/>). In the simulated biodegradation tree, the following *model categories* were assigned to every reaction rule to estimate the likelihood of each identified reaction type under the specified conditions: (i) very likely reaction, corresponding to the reactions that almost certainly occur and occur with the highest priority; (ii) likely reaction, used when almost all microbes can catalyze a given reaction with a functional group present in a molecule; (iii) neutral, applicable to reactions that are common but not certainly occur in each simulated system. Some reactions, for instance, which are generally quite possible, may or may not be likely to occur for each substrate and require individual investigation. Some reactions from “neutral” category may thus be “likely”, some may be “possible” and some may be “unlikely”, based on current knowledge. The likelihood thresholds for carbendazim biodegradation modelling were set (and colour coded) as follows: (i) 0 – 0.2 for very unlikely reactions (red); (ii) 0.3 – 0.4 for unlikely reactions (orange); (iii) 0.5 – 0.6 for possible reactions (yellow for 0.5 and light green for 0.6); (iv) 0.7 – 0.8 for likely reactions (green) and (v) 0.9 – 1.0 for very likely reactions (dark green).

Selection of the **stop criteria** finishing the simulation procedure performed by CRAFT Explorer and resulting in the amount of reactions and products generated required specification of the number of biodegradation steps, i.e. the number of levels of biodegradation, that will be considered by the software during the simulation procedure.

Increasing the number of the degradation steps increases the number of identified products since the procedure of submitting all molecules (i.e. products generated at each degradation level) is repeated. For the purpose of carbendazim biodegradation simulation the maximal number of steps (i.e. 5) was selected, in order to perform the modelling in the broadest possible sense.

The last step before final biodegradation simulation was the selection of **overall likelihood threshold**. The overall likelihood of a reaction product can be defined, in terms of CRAFT Explorer, as a product of the likelihood of all the preceding products in the degradation tree (Figure 2).



**Figure 2.** The CRAFT Explorer 1.0 overall likelihood threshold definition

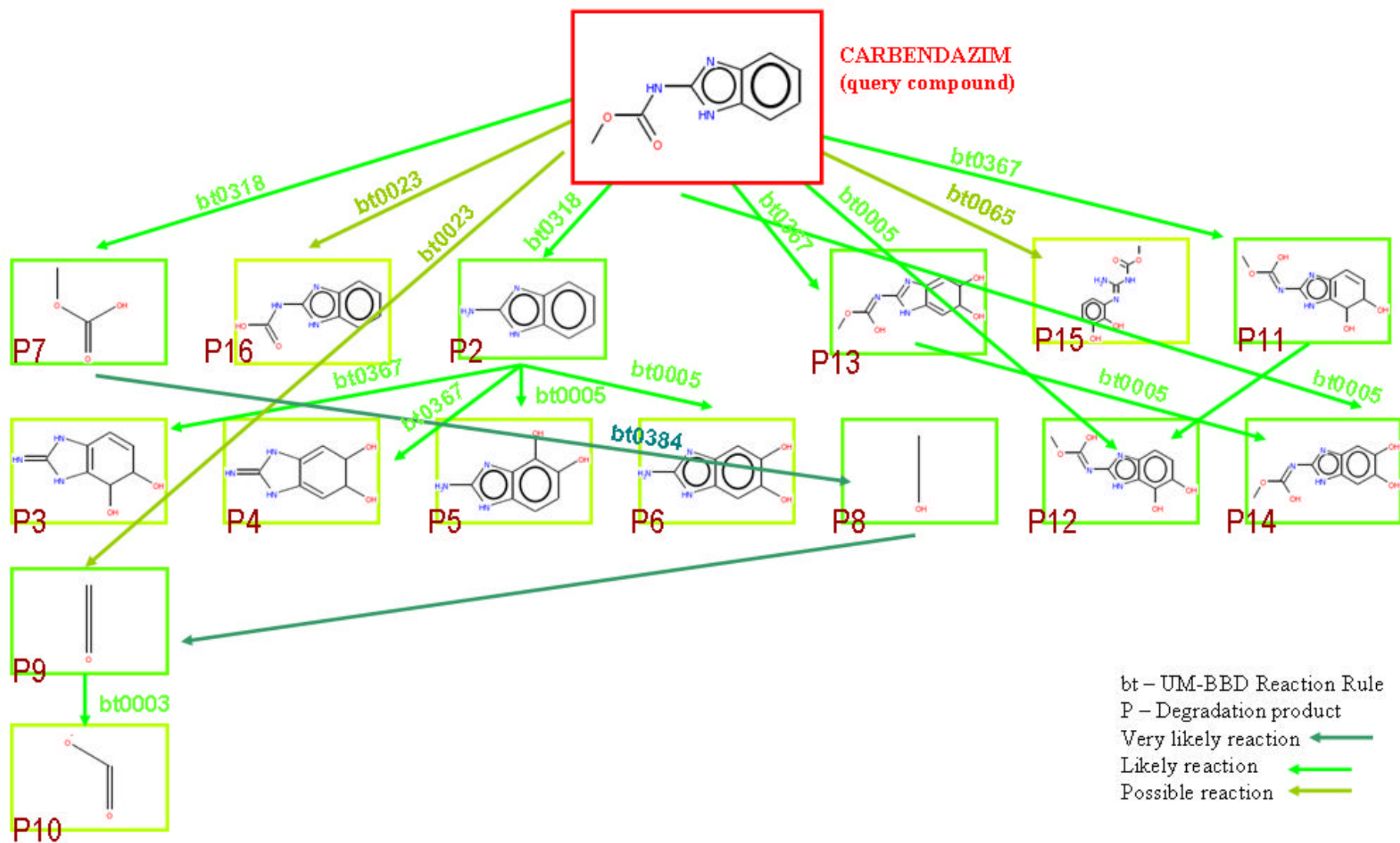
Selection of the overall likelihood threshold allows the generation of the less likely products by CRAFT to be reduced. The higher is the threshold, fewer products will be identified by the software. The threshold was set as 0.6, thus CRAFT Explorer has not generated products with the lower value of the overall likelihood.

### 3.2. Carbendazim biodegradation tree

Carbendazim biodegradation tree, along with the structures of generated products (metabolites), is visualised in Figure 3. The diagram shows the entire degradation pathway, including all degradation routes leading to each identified degradation product. The arrows indicate the single reactions and the colours indicate their likelihood. The structure of each generated molecule (starting with the query compound carbendazim situated at the top of the diagram) is represented in a box, located on the level corresponding to the level of biodegradation.

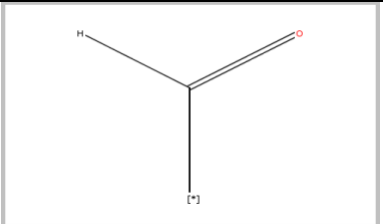
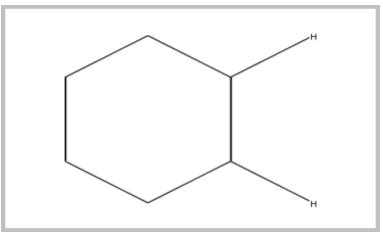
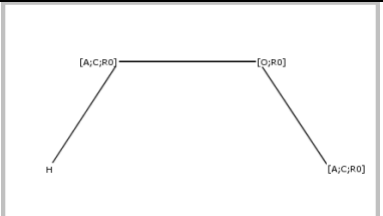
The biodegradation pathway of carbendazim consists of 17 degradation steps leading to the formation of 15 various degradation products (P2 – P16). All metabolites are formed as a result of particular reaction types associated with particular UM-BBD reaction rules – the total amount of both, identified by CRAFT, was 7. The detailed list and description of reactions, along with “active” substructural fragments of given substrates, is given in Table 2. The overview of generated products is given in Table 3.

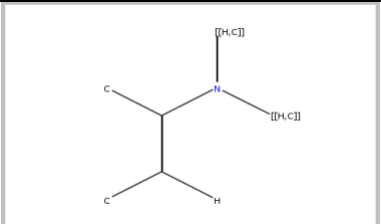

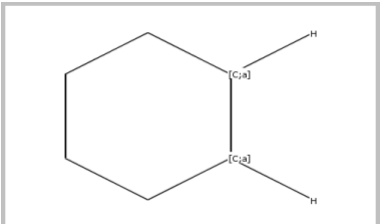
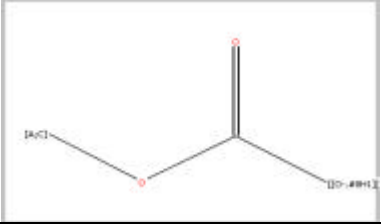
In general, the most frequent reaction types, each leading to the formation of 4 various degradation products at different degradation levels were UM-BBD bt0005 (vic-unsubstituted aromatic  $\rightleftharpoons$  vic-dihydroxyaromatic) and UM-BBD bt0367 (vic-unsubstituted aromatic  $\rightleftharpoons$  vic-dihydrodihydroxyaromatic), both connected with dioxygenation of vic-unsubstituted aromatics and subsequent oxidation, leading to the formation of catechol derivatives.



**Figure 3.** Carbendazim biodegradation tree simulated by CRAFT Explorer 1.0

**Table 2.** Reaction rules and reaction types corresponding with particular structural alerts identified by CRAFT Explorer 1.0 during the simulation of carbendazim biodegradation pathway (UM-BBD, <http://umbbd.msi.umn.edu/>)

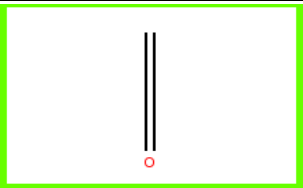
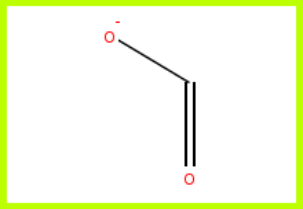
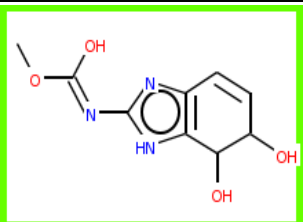
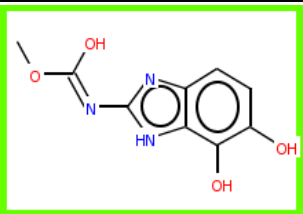
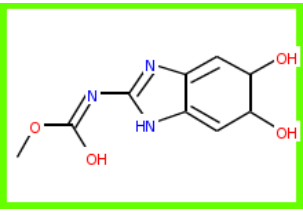
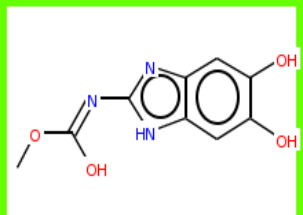
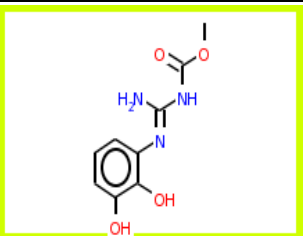
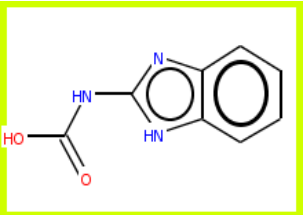
No	UM-BBD Reaction Rule	Reaction Type	Associated Structural Alert	Comments
1	UM-BBD bt0003	Aldehyde $\rightleftharpoons$ Carboxylate		Likely reaction.
2	UM-BBD bt0005	vic-unsubstituted Aromatic $\rightleftharpoons$ vic-Dihydroxyaromatic		Likely reaction.  This rule handles the dioxygenation of vic-unsubstituted aromatics and subsequent oxidation to form the catechol derivative. When there is more than one potential site of dioxygenation, all possible positions are given. This rule acts on both cis and trans-dihydrodiol intermediates. The aromatic hydrocarbon dioxygenases produce an activated dioxygen species that is thought to be sufficiently reactive to potentially functionalize most aromatic ring carbon atoms. Commonly, the position of dioxygenation is determined by the orientation of the ring(s) in the enzyme's active site, which cannot be predicted. All fused aromatic ring products with hydroxyl at 2,3 position are excluded. This rule does not act on heterocyclic aromatic rings and many aromatic carboxylates.
3	UM-BBD bt0023	dialiphatic Ether $\rightleftharpoons$ Alcohol + Aldehyde		Neutral reaction.  This rule does not handle ethers when the vicinal carbons are halogenated. When given an aromatic aliphatic ether (Ar-O-R <sub>1</sub> ) it will produce ArOH + R <sub>1</sub> CHO. When given a dialiphatic ether (R <sub>1</sub> -O-R <sub>2</sub> ) it will produce both R <sub>1</sub> -OH + R <sub>2</sub> CHO and R <sub>2</sub> -OH + R <sub>1</sub> -CHO. It will not act on a diaromatic ether (Ar <sub>1</sub> -O-Ar <sub>2</sub> ) or a ring ether. Rule is blocked from acting on CoA.

No	UM-BBD Reaction Rule	Reaction Type	Associated Structural Alert	Comments
4	UM-BBD bt0065	1-Amino-2-unsubstituted aromatic $\approx$ vic-Dihydroxyaromatic + Amine		Neutral reaction.  This rule also handles fused rings. All fused aromatic ring products with hydroxyl at 2,3 position are excluded.
5	UM-BBD bt0318	Carbamyl $\approx$ Amine + Carbonate		Likely reaction.
6	UM-BBD bt0367	vic-unsubstituted Aromatic $\approx$ vic-Dihydrodihydroxyaromatic		Likely reaction.  This rule handles the dioxygenation of vic-unsubstituted aromatics and subsequent oxidation to form the catechol derivative. When there is more than one potential site of dioxygenation, all possible positions are given. This rule acts on both cis and trans-dihydrodiol intermediates. The aromatic hydrocarbon dioxygenases produce an activated dioxygen species that is thought to be sufficiently reactive to potentially functionalize most aromatic ring carbon atoms. Commonly, the position of dioxygenation is determined by the orientation of the ring(s) in the enzyme's active site, which cannot be predicted. All fused aromatic ring products with hydroxyl at 2,3 position are excluded. This rule does not act on heterocyclic aromatic rings and many aromatic carboxylates.
7	UM-BBD bt0384	mono Alkylcarbonate $\approx$ Alkyl 1-ol		Very likely reaction.



**Table 3.** The overview of carbendazim biodegradation products simulated by CRAFT Explorer 1.0

	<p><b>CRAFT ID: P2</b>  <b><u>2-aminobenzimidazole (2-AB)</u></b>            CRAFT overall likelihood: 0.80 (Likely)            SMILES: <chem>C=1C=CC=2NC(=NC=2(C=1))N</chem>            CRAFT shortest pathway:  <b>Carbendazim</b> <math>\approx</math> <b>UM-BBD bt0318</b> <math>\approx</math> <b>P2</b></p>
	<p><b>CRAFT ID: P3</b>  <b><u>2-imino-2,3-dihydro-1H-benzimidazole-4,5-diol</u></b>            CRAFT overall likelihood: 0.64 (Likely)            SMILES: <chem>N=C1NC=2C=CC(O)C(O)C=2(N1)</chem>            CRAFT shortest pathway:  <b>Carbendazim</b> <math>\approx</math> <b>UM-BBD bt0318</b> <math>\approx</math> <b>P2</b> <math>\approx</math> <b>UM-BBD bt0367</b> <math>\approx</math> <b>P3</b></p>
	<p><b>CRAFT ID: P4</b>  <b><u>2-imino-2,3-dihydro-1H-benzimidazole-5,6-diol</u></b>            CRAFT overall likelihood: 0.64 (Likely)            SMILES: <chem>N=C1NC2=CC(O)C(O)C=C2(N1)</chem>            CRAFT shortest pathway:  <b>Carbendazim</b> <math>\approx</math> <b>UM-BBD bt0318</b> <math>\approx</math> <b>P2</b> <math>\approx</math> <b>UM-BBD bt0367</b> <math>\approx</math> <b>P4</b></p>
	<p><b>CRAFT ID: P5</b>  <b><u>2-AB-4,5-diol</u></b>            CRAFT overall likelihood: 0.64 (Likely)            SMILES: <chem>OC=1C=CC=2NC(=NC=2(C=1(O)))N</chem>            CRAFT shortest pathway:  <b>Carbendazim</b> <math>\approx</math> <b>UM-BBD bt0318</b> <math>\approx</math> <b>P2</b> <math>\approx</math> <b>UM-BBD bt0005</b> <math>\approx</math> <b>P5</b></p>
	<p><b>CRAFT ID: P6</b>  <b><u>2-AB-5,6-diol</u></b>            CRAFT overall likelihood: 0.64 (Likely)            SMILES: <chem>OC1=CC=2N=C(N)NC=2(C=C1(O))</chem>            CRAFT shortest pathway:  <b>Carbendazim</b> <math>\approx</math> <b>UM-BBD bt0318</b> <math>\approx</math> <b>P2</b> <math>\approx</math> <b>UM-BBD bt0005</b> <math>\approx</math> <b>P6</b></p>
	<p><b>CRAFT ID: P7</b>  <b><u>Monomethyl carbonate</u></b>            CRAFT overall likelihood: 0.80 (Likely)            SMILES: <chem>COC(=O)O</chem>            CRAFT shortest pathway:  <b>Carbendazim</b> <math>\approx</math> <b>UM-BBD bt0318</b> <math>\approx</math> <b>P7</b></p>
	<p><b>CRAFT ID: P8</b>  <b><u>Methanol</u></b>            CRAFT overall likelihood: 0.80 (Likely)            SMILES: <chem>CO</chem>            CRAFT shortest pathway:  <b>Carbendazim</b> <math>\approx</math> <b>UM-BBD bt0318</b> <math>\approx</math> <b>P7</b> <math>\approx</math> <b>UM-BBD 0384</b> <math>\approx</math> <b>P8</b></p>

	<p><b>CRAFT ID: P9</b>  <b>Formaldehyde</b>  CRAFT overall likelihood: 0.80 (Likely)  SMILES: C=O  CRAFT shortest pathway:  <b>Carbendazim</b> <math>\approx</math> <b>UM-BBD bt0023</b> <math>\approx</math> <b>P9</b></p>
	<p><b>CRAFT ID: P10</b>  <b>Formic acid</b>  CRAFT overall likelihood: 0.64(Likely)  SMILES: O=C[O-]  CRAFT shortest pathway:  <b>Carbendazim</b> <math>\approx</math> <b>UM-BBD bt0023</b> <math>\approx</math> <b>P9</b> <math>\approx</math> <b>UM-BBD bt0003</b> <math>\approx</math> <b>P10</b></p>
	<p><b>CRAFT ID: P11</b>  <b>Methyl hydrogen (6,7-dihydroxy-6,7-dihydro-1H-benzimidazol-2-yl)imidocarbonate</b>  CRAFT overall likelihood: 0.80 (Likely)  SMILES: COC(O)=NC1=NC=2C=CC(O)C(O)C=2(N1)  CRAFT shortest pathway:  <b>Carbendazim</b> <math>\approx</math> <b>UM-BBD bt0367</b> <math>\approx</math> <b>P11</b></p>
	<p><b>CRAFT ID: P12</b>  <b>Methyl hydrogen (6,7-dihydroxy-1H-benzimidazol-2-yl)imidocarbonate</b>  CRAFT overall likelihood: 0.80 (Likely)  SMILES: COC(O)=NC1=NC=2C=CC(O)=C(O)C=2(N1)  CRAFT shortest pathway:  <b>Carbendazim</b> <math>\approx</math> <b>UM-BBD bt0005</b> <math>\approx</math> <b>P12</b></p>
	<p><b>CRAFT ID: P13</b>  <b>Methyl hydrogen (5,6-dihydroxy-5,6-dihydro-1H-benzimidazol-2-yl)imidocarbonate</b>  CRAFT overall likelihood: 0.80 (Likely)  SMILES: COC(O)=NC1=NC2=CC(O)C(O)C=C2(N1)  CRAFT shortest pathway:  <b>Carbendazim</b> <math>\approx</math> <b>UM-BBD bt0367</b> <math>\approx</math> <b>P13</b></p>
	<p><b>CRAFT ID: P14</b>  <b>Methyl (5,6-dihydroxy-1H-benzimidazol-2-yl)carbamate (5,6-DHCB)</b>  CRAFT overall likelihood: 0.80 (Likely)  SMILES: COC(O)=NC1=NC2=CC(O)=C(O)C=C2(N1)  CRAFT shortest pathway:  <b>Carbendazim</b> <math>\approx</math> <b>UM-BBD bt0005</b> <math>\approx</math> <b>P14</b></p>
	<p><b>CRAFT ID: P15</b>  <b>((E)-aminof(2,3-dihydroxyphenyl)dimino)methylcarbamic acid</b>  CRAFT overall likelihood: 0.60 (Possible)  SMILES: COC(=O)NC(=NC=1C=CC=C(O)C=1(O))N  CRAFT shortest pathway:  <b>Carbendazim</b> <math>\approx</math> <b>UM-BBD bt0065</b> <math>\approx</math> <b>P15</b></p>
	<p><b>CRAFT ID: P16</b>  <b>1H-benzimidazol-2-ylcarbamic acid (2-Benzimidazolecarbamic acid)</b>  CRAFT overall likelihood: 0.60 (Possible)  SMILES: O=C(O)NC1=NC=2C=CC=CC=2(N1)  CRAFT shortest pathway:  <b>Carbendazim</b> <math>\approx</math> <b>UM-BBD bt0023</b> <math>\approx</math> <b>P16</b></p>

## 4. Simulating mammalian metabolism of carbendazim with Meteor

### 4.1. Meteor methodology and model details

The mammalian metabolic fate of a fungicide carbendazim was simulated by Meteor v. 12.0.0 software tool of Lhasa Ltd. (<https://www.lhasalimited.org/index.php/meteor/>). The simulation was based on the expert knowledge rules in metabolism organised and stored in a knowledge base (database version used: Meteor12.0.0\_14\_08\_2009). The query molecular structure of carbendazim was processed against biotransformations and reasoning rules from the Meteor knowledge base. The predictions were performed with respect to mammals and did not grow from metabolites issued from phase II biotransformations. The number of generated metabolites was limited (as default) to 400 and the maximum number of steps generated in any given pathway was 4.

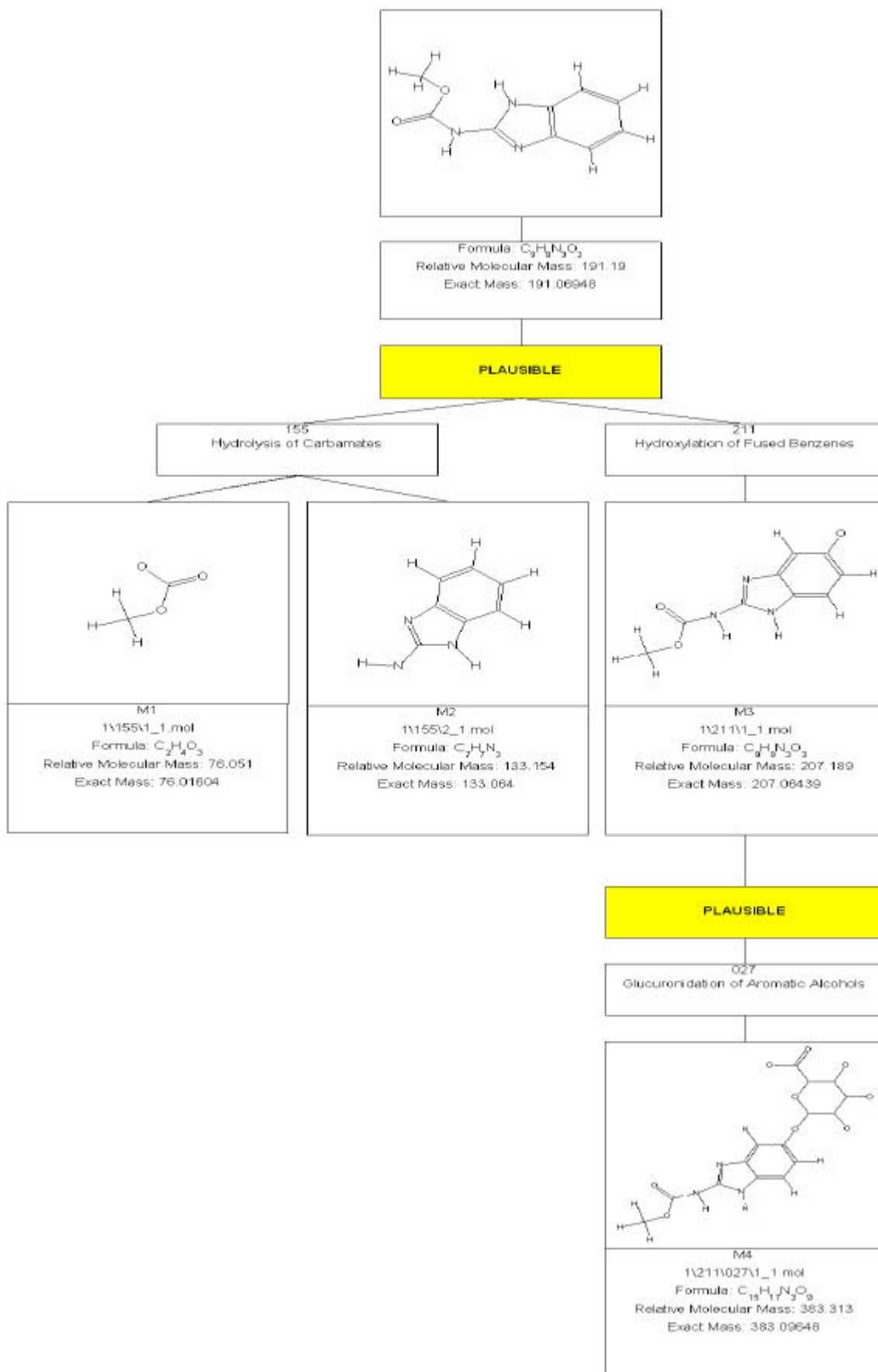
The procedure of generating carbendazim biotransformations consisted of three steps:

- (i) searching the knowledge database for biotransformations and populating a list of potential metabolites on the basis of carbendazim molecular structure;
- (ii) eliminating generated metabolites according to the absolute reasoning rules, which are based on factors such as observed rates of occurrence and yields in the literature, the physicochemical properties of the substrate (Log P and molecular weight) and whether a substrate is the query structure or a metabolite generated by Meteor, thereby selecting metabolites with a specified level of likelihood and compiling a shorter list of metabolites. The uncertainty terms used in Meteor in relation to predicted biotransformations and metabolites include: “probable” (when there is at least one strong argument that the proposition is true and there are no arguments against it), “plausible” (when the weight of evidence supports the proposition), “equivocal” (when there is an equal weight of evidence for and against the proposition), “doubted” (when the weight of evidence opposes the proposition) and “improbable” (when there is at least one strong argument that the proposition is false and there are no arguments that it is true). For the purpose of the present investigation, the “plausible” level of likelihood was selected (biotransformations adjudged to be at least plausible are displayed);
- (iii) eliminating more metabolites, according to the relative reasoning rules, by comparing competitive biotransformations and deciding which will take precedence. The relative reasoning included only the most likely (so-called “level 1”) biotransformations.

On completion of the Meteor analysis, the knowledge base biotransformations matching the query structure were visualised in the form of a metabolic tree.

### 4.2. Carbendazim metabolic tree

The metabolic tree of carbendazim (Figure 4), presenting Meteor 12.0.0 simulation results, begins with the query structure (Q) situated at the top and followed by first generation metabolites (M1-M3) and, subsequently, by further generations of metabolites below their respective parents (M4). The tree visualises the whole pathway leading from carbendazim to metabolites via available biotransformations. In the tree hierarchy the information on the likelihood levels, indicating the likelihood of the first and subsequent generation biotransformations occurring, is also provided and colour coded.

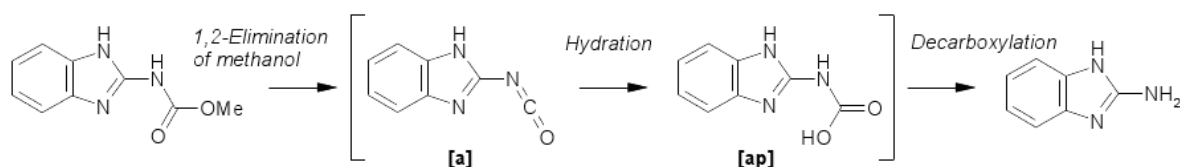


**Figure 4.** Carbendazim metabolic tree simulated by Meteor 12.0.0

Meteor 12.0.0 identified two types of phase I biotransformations that carbendazim undergoes inside mammalian organism, namely: the hydrolysis of carbamates and the hydroxylation of fused benzenes and one type of phase II biotransformation, namely the glucuronidation of aromatic alcohols. Each biotransformation was identified by a knowledge base reference number and name; the general overview of all of them is provided in Table 4.

The hydrolysis of carbamates as well as the hydroxylation of fused benzenes were considered as reactions likely to occur in mammals. The generation of metabolites M1-M3 resulting from both phase I reaction types was estimated as “plausible”. This likelihood was assessed on the basis of carbendazim lipophilicity, according to general principles described by Testa & Cruciani (2001). It was also dependent on whether the considered molecule was an administered (query) compound (i.e. carbendazim in this case) or a metabolite generated by the software (Hutson, 1989). Phase I biotransformations #155 and #211 were estimated “plausible”, as carbendazim was the substrate of medium lipophilicity (otherwise the likelihood associated with considered reactions would be “doubted”) and was an administered (query) compound (if the substrate was a generated metabolite, the likelihood would be lower).

Meteor gives an additional option to screen the intermediates in particular biotransformation reactions. For carbamate hydrolysis reaction two intermediates (namely isocyanate and carbamic acid) can be expressed and reported by the software (but they are not subjected to the effects of the reasoning engine or separate processing). Isocyanate belongs to “potentially adduct-forming” intermediates (Meteor type-a intermediates), due to its expected high electrophilicity. Carbamic acid is additionally labelled as “presumed” intermediate (Meteor type-ap), as it decarboxylates extremely rapidly, is difficult to characterise and is not expected to accumulate or be detected (Figure 5).



**Figure 5.** Intermediates in hydrolysis of carbamates reaction given by Meteor 12.0.0

As far as the hydroxylation of fused benzene compounds is concerned, N additional relative reasoning rule, stating that the reaction is more likely in the 2(3) position than in the 1(4) position, was applied during metabolite generation.

The substructural fragments of carbendazim contributing to particular phase I reactions as well as the details (including names, SMILES strings and lipophilicity data) concerning all generated metabolites are provided in Table 5.

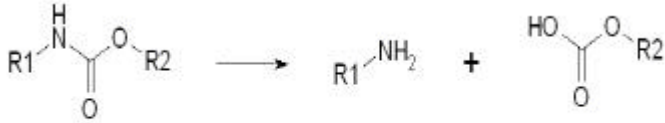
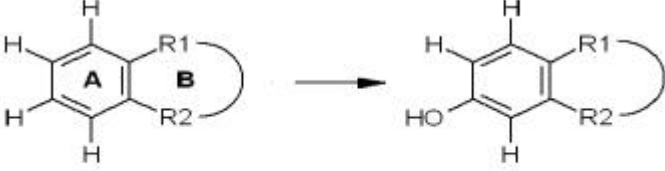
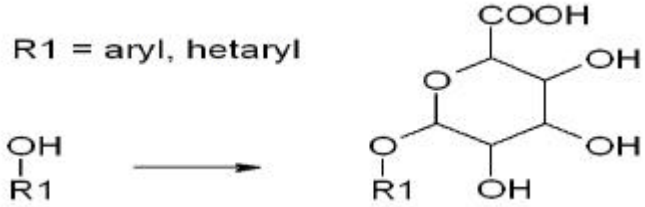
As far as phase II biotransformation is concerned, one reaction type (glucuronidation of aromatic alcohols) was identified by Meteor 12.0.0. Its likelihood was considered purely dependent on the lipophilicity of the substrate according to general principles only (Testa & Cruciani, 2001) and was deemed independent of whether the substrate was an administered (query) compound or a generated metabolite. As the lipophilicity of M3 (a substrate in this case) was medium, the reaction was estimated as “plausible” (prominent phase II reactions

such as glucuronidation are typically considered as "probable" for substrates of high lipophilicity and "equivocal" for those of low lipophilicity) (Testa & Cruciani, 2001).

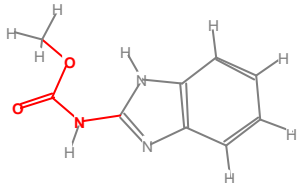
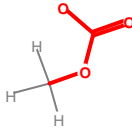
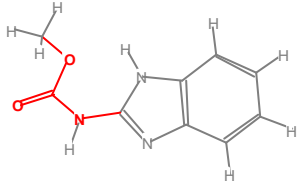
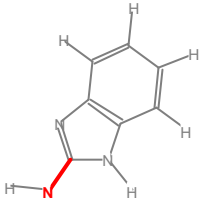
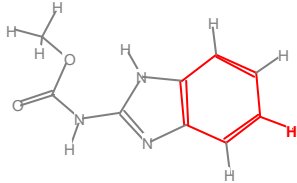
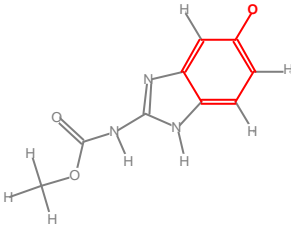
Additionally (at the step of relative reasoning), the reaction of aromatic alcohols (phenols and heteroaromatic analogues) sulphation was considered as competitive to glucuronidation, but finally turned out to be less likely.

The details concerning carbendazim phase II biotransformation and the resulting metabolite (M4) are presented in Table 6.

**Table 4.** The overview of carbendazim biotransformations predicted by Meteor 12.0.0

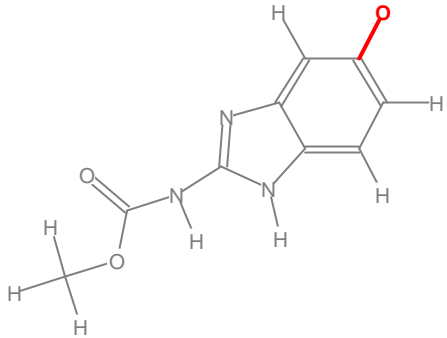
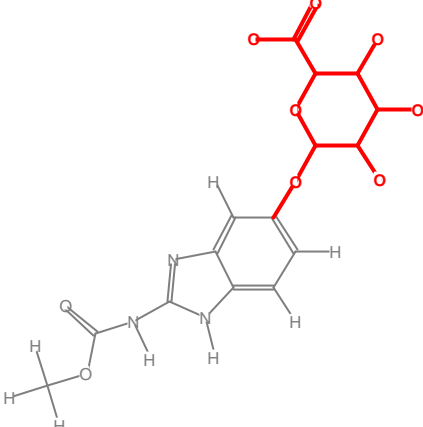
Meteor Biotransformation No (Phase, enzyme)	Biotransformation Name (Parent $\rightleftharpoons$ Metabolite)	Reaction	General Comments (about the scope and limitations of the biotransformation)
#155 (Phase I, Hydrolase)	Hydrolysis of Carbamates  (Carbendazim $\rightleftharpoons$ M1) (Carbendazim $\rightleftharpoons$ M2)	 <p>R1, R2 = aliphatic or aromatic carbon</p>	Hydrolysis of carbamate esters has been demonstrated for a fairly wide range of substrates. In general the reaction is slower than for the corresponding carboxylic amide.
#211 (Phase I, CYP450)	Hydroxylation of Fused Benzenes  (Carbendazim $\rightleftharpoons$ M3)	 <p>R1/R2 = Any atom type except Carbon Ring B = Aliphatic or Aromatic</p>	Phenolic compounds are the most stable and frequently excreted products of aromatic ring hydroxylation mediated by cytochrome P450 enzymes (other products may be epoxides, dihydrodiols, catechols or quinones). The mechanisms involved are complex and the product regioselectivity is difficult to predict.
#027 (Phase II, UGT)	Glucuronidation of Aromatic Alcohols  (M3 $\rightleftharpoons$ M4)	<p>R1 = aryl, hetaryl</p> 	The formation of glucuronides from phenols is an important reaction in drug metabolism. The position of hydroxy groups relative to other ring substituents does not significantly affect conjugation. Likewise, bulky ortho- (and di-ortho) substituents do not hinder conjugation in some species. The donating coenzyme for the reaction is uridine-5'-diphospho-alpha-D-glucuronic acid. Glucuronic acid has the alpha-configuration when bound in the coenzyme but has the beta-configuration when bound as a glycone. The carboxylic acid function in glucuronides has a pKa of 3.5 - 4.0 which means that they are essentially fully ionised in plasma and urine. The combined effects of the carboxylate anion and hydroxyl group account for the extreme polarity, lack of biological activity and ready urinary excretion of most glucuronides. High molecular weight glucuronides may enter an enterohepatic circulation pathway via biliary excretion and reversion to the aglycone through the action of gut microflora beta - glucuronidases.

**Table 5.** Phase I metabolites of carbendazim generated by Meteor 12.0.0 (structural alerts marked in red). Bt (Biotransformation) #155 refers to the hydrolysis of carbamates and #211 to the hydroxylation of fused benzenes

Parent molecule	Meteor Bt Number (Enzyme)	Metabolite
	#155 (Hydrolase)	
<p><b>Q: Carbendazim (MCB)</b>  <b>SMILES:</b>  <chem>c12c(cccc1)N=C(NC(=O)OC)N2</chem>  <b>Log P:</b> 1.709 (from external data source used to calculate Log P)</p>		<p><b>M1: Monomethyl carbonate</b>  <b>SMILES:</b>  <chem>COC(=O)O</chem>  <b>Log P:</b> -0.869 (from external data source used to calculate Log P)</p>
	#155 (Hydrolase)	
<p><b>Q: Carbendazim (MCB)</b>  <b>SMILES:</b>  <chem>c12c(cccc1)N=C(NC(=O)OC)N2</chem>  <b>Log P:</b> 1.709 (from external data source used to calculate Log P)</p>		<p><b>M2: 2-aminobenzimidazole (2-AB)</b>  <b>SMILES:</b>  <chem>C=1C=CC=2NC(=NC=2(C=1))N</chem>  <b>Log P:</b> 1.238 (from external data source used to calculate Log P)</p>
	#211 (CYP450)	
<p><b>Q: Carbendazim (MCB)</b>  <b>SMILES:</b>  <chem>c12c(cccc1)N=C(NC(=O)OC)N2</chem>  <b>Log P:</b> 1.709 (from external data source used to calculate Log P)</p>		<p><b>M3: Methyl (5-hydroxybenzimidazol-2-yl)carbamate (5-HBC)</b>  <b>SMILES:</b>  <chem>c12c(cc(O)cc1)N=C(NC(=O)OC)N2</chem>  <b>Log P:</b> 1.757 (from external data source used to calculate Log P)</p>



**Table 6.** Phase II metabolites of carbendazim generated by Meteor 12.0.0 (structural alerts marked in red). Bt (Biotransformation) #027 refers to the glucuronidation of aromatic alcohols

Parent molecule	Meteor Bt Number (Enzyme)	Metabolite
	<p>#027 (UGT)</p> <p>➔</p>	
<p><b><u>M3: Methyl (5-hydroxybenzimidazol-2-yl)carbamate (5-HBC)</u></b>  <b><u>SMILES:</u></b>  <chem>c12c(cc(O)cc1)N=C(NC(=O)OC)N2</chem>  <b>Log P:</b> 1.757 (from external data source used to calculate Log P)</p>		<p><b><u>M4: Glucuronic acid conjugate of Methyl 5-hydroxy-1H-benzimidazol-2-ylcarbamate (5-HBC-G)</u></b>  <b><u>SMILES:</u></b>  <chem>c12c(cc(OC3C(O)C(O)C(O)C(C(=O)O)O3)cc1)N=C(NC(=O)OC)N2</chem>  <b>Log P:</b> -0.547 (from external data source used to calculate Log P)</p>

## 5. Predicting carbendazim metabolites with the QSAR Application Toolbox

Two metabolism simulators (microbial and liver mammalian) are implemented in the OECD QSAR Application Toolbox 2.0 (beta version). The microbial metabolism simulator is based on the original CATABOL simulator (Dimitrov et al., 2004; Dimitrov et al., 2002; Jaworska et al., 2002). The liver mammalian metabolism simulator is based on the methodology applied in OASIS TIMES (Tissue Metabolism Simulator) software. CATABOL and TIMES are developed and marketed by the Laboratory of Mathematical Chemistry (<http://www.oasis-lmc.org/?section=software/>).

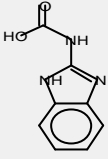
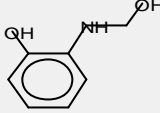
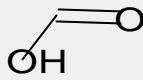
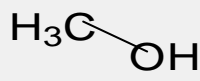
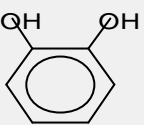
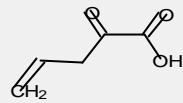
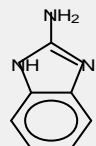
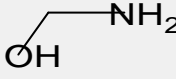
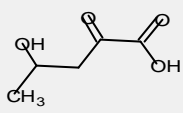
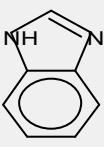

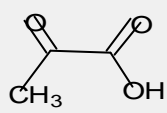
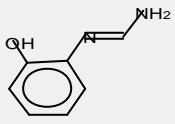
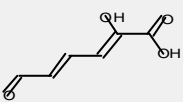
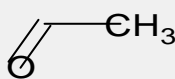
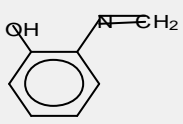
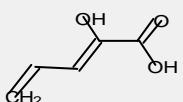
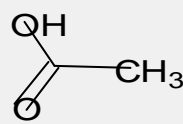
The publicly available OECD QSAR Application Toolbox does not provide insights into the detailed maps of microbial/mammalian biotransformations, which is possible in the original (commercial) models. Nevertheless, both simulators allow the user to predict the sets of possible metabolites, resulting either from microbial activity or from biotransformation reactions occurring in the liver of different mammals. In this study, the simulation of metabolites by the OECD software was used for comparative purposes.

The simulations were performed on the basis of experimental data, stored in two databases: (i) **"observed microbial catabolism"** database, including information on aerobic catabolism for 200 diverse chemicals (C1-compounds, aliphatic hydrocarbons, alicyclic rings, furans, halogenated hydrocarbons, aromatic hydrocarbons and haloaromatics, amines, sulphonates, nitrates, nitro-derivatives, nitriles and compounds containing more than one functional group) and compiled from scientific literature as well as publicly available databases, e.g. the University of Minnesota Biocatalysis and Biodegradation Database (Ellis et al., 2006); (ii) **"observed mammalian metabolism"** database including documented *in vivo* and *in vitro* information on metabolic pathways in mammalian microsomes for 200 diverse chemicals (including chemicals with various functionalities, aliphatic hydrocarbons, alicyclic rings, furans, halogenated hydrocarbons, aromatic hydrocarbons and haloaromatics, amines, sulphonates, nitrates, nitro-derivatives), also retrieved from scientific publications and public web sites.

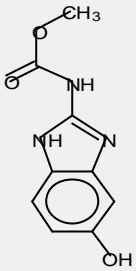
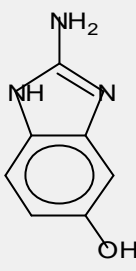
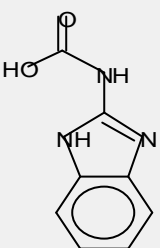
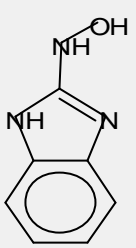
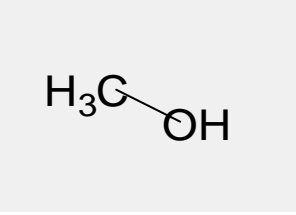
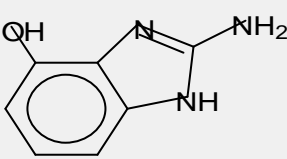
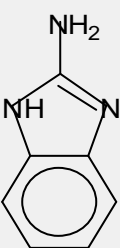
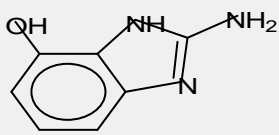
The details of methodology implemented in both simulators are described in detail in the original CATABOL (Dimitrov et al., 2004; Dimitrov et al., 2002; Jaworska et al., 2002) and TIMES (Mekenyan et al., 2004) papers. For the purpose of populating the reasonable number of metabolites, the software settings were kept as default, i.e.: (i) for microbial metabolism only the first level of biodegradation with transformations having minimal probability of 0.001 was simulated; (ii) for mammalian liver metabolism 5 levels of biodegradation and reactions with the minimal probability equal 0.7 were considered. These constraints were suggested by the developers, as relating the simulators with the reality in the best way, and were based on experts knowledge as well as available experimental data.

The microbial metabolism simulation resulted in the formation of 18 potential carbendazim degradation products (Table 7). As far as liver metabolism of carbendazim is concerned, the OECD software provided a set of 8 metabolites (Table 8). The results were highly dependent on the user constraints. For instance, changing the thresholds for microbial metabolism simulation and considering 5 degradation levels with minimal probability of each reaction equal 0.6 (as in CRAFT Explorer) resulted in the generation of 813 different carbendazim degradation products.

**Table 7.** Microbial metabolites of carbendazim predicted by the OECD QSAR Application Toolbox 2.0 (beta version)

#	Metabolite	#	Metabolite	#	Metabolite
1	 <chem>c12c(cccc1)N=C(NC(=O)O)N2</chem> <b><u>1H-benzimidazol-2-ylcarbamic acid</u></b> <b><u>(2-Benzimidazolecarbamic acid)</u></b>	7	 <chem>c1(O)c(NCO)cccc1</chem> <b><u>2-[(hydroxymethyl)amino]phenol</u></b>	13	 <chem>C(=O)O</chem> <b><u>Formic acid</u></b>
2	 <chem>CO</chem> <b><u>Methanol</u></b>	8	 <chem>c1(O)c(O)cccc1</chem> <b><u>Catechol</u></b>	14	 <chem>C(=O)(O)C(=O)CC=C</chem> <b><u>2-oxopent-4-enoic acid</u></b>
3	 <chem>c12c(cccc1)N=C(N)N2</chem> <b><u>2-aminobenzimidazole</u></b> <b><u>(2-AB)</u></b>	9	 <chem>C(N)O</chem> <b><u>Aminomethanol</u></b>	15	 <chem>C(=O)(O)C(=O)CC(O)C</chem> <b><u>4-hydroxy-2-oxopentanoic acid</u></b>
4	 <chem>c12c(cccc1)N=CN2</chem> <b><u>Benzimidazole</u></b>	10	 <chem>C=O</chem> <b><u>Formaldehyde</u></b>	16	 <chem>C(=O)(O)C(=O)C</chem> <b><u>Pyruvic acid</u></b> <b><u>(2-oxopropanoic acid)</u></b>
5	 <chem>c1(O)c(N=CN)cccc1</chem> <b><u>N'-(2-hydroxyphenyl)imidoforamide</u></b>	11	 <chem>C(=O)(O)C(O)=CC=CC=O</chem> <b><u>2-hydroxy-6-oxohexa-2,4-dienoic acid</u></b>	17	 <chem>C(C)=O</chem> <b><u>Acetaldehyde</u></b>
6	 <chem>c1(O)c(N=C)cccc1</chem> <b><u>2-(methylideneamino)phenol</u></b>	12	 <chem>C(=O)(O)C(O)=CC=C</chem> <b><u>2-hydroxy-2,4-pentadienoic acid</u></b>	18	 <chem>C(C(=O)O)C</chem> <b><u>Acetic acid</u></b>

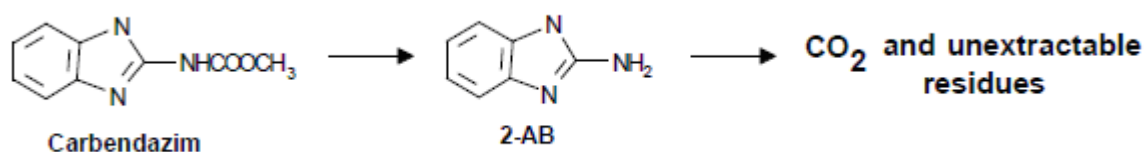
**Table 8.** Liver mammalian metabolites of carbendazim predicted by the OECD QSAR Application Toolbox 2.0 (beta version)

#	Metabolite	#	Metabolite
1	 <chem>c12c(cc(O)cc1)N=C(NC(=O)OC)N2</chem> <b><u>5-HBC</u></b>	5	 <chem>c12c(cc(O)cc1)N=C(N)N2</chem> <b><u>2-aminobenzimidazol-5-ol</u></b>
2	 <chem>c12c(ccc1)N=C(NC(=O)O)N2</chem> <b><u>1H-benzimidazol-2-ylcarbamic acid</u></b> <b><u>(2-Benzimidazolecarbamic acid)</u></b>	6	 <chem>c12c(ccc1)N=C(NO)N2</chem> <b><u>N-hydroxy-1H-benzimidazol-2-amine</u></b>
3	 <chem>CO</chem> <b><u>Methanol</u></b>	7	 <chem>c1(O)c2c(ccc1)NC(N)=N2</chem> <b><u>2-aminobenzimidazol-4-ol</u></b>
4	 <chem>c12c(ccc1)N=C(N)N2</chem> <b><u>2-aminobenzimidazol (2-AB)</u></b>	8	 <chem>c1(O)c2c(ccc1)N=C(N)N2</chem> <b><u>2-aminobenzimidazol-7-ol</u></b>

## 6. Discussion and conclusions

### 6.1. Biodegradation of carbendazim

It is difficult to evaluate the results of the biodegradation simulations with CRAFT and the QSAR Application Toolbox, due to the paucity of experimental data. According to the scarce information available in the scientific literature, carbendazim is decomposed in the environment with half-lives of 6 to 12 months in bare soil, 3 to 6 months in turf, and half-lives in water of 2 and 25 months under aerobic and anaerobic conditions, respectively. The major microbial metabolism product is 2-aminobenzimidazole (2-AB), which undergoes further biodegradation (Figure 6). Another possible degradation product suggested is 1,2-diaminobenzene (JMPR, 1999), this was however not predicted by any of the applied software.



**Figure 6.** Biodegradation pathway of carbendazim suggested in the literature (JMPR, 1999)

Both software tools, namely CRAFT Explorer 1.0 and OECD QSAR Application Toolbox, predicted 2-AB as a major compound formed due to microbial activity and the possible products of its subsequent decomposition (e.g. CRAFT P3, P4, P5, P6, all being "diol" derivatives). At the same time, both tools identified many more products that could theoretically be generated (depending on the reaction conditions), as illustrated in tables 3 and 7.

In the context of a chemical risk assessment, the CRAFT predictions, suggesting the main biodegradation routes and products of carbendazim, could be used to guide further investigations. It is not possible to conclude whether the simulated information is adequate to fill current data gaps (e.g. concerning the aerobic biodegradation of carbendazim in soil), since ultimately this is a policy decision. The simulations do however raise a number of questions that should be considered when judging adequacy in a regulatory context, such as:

- how constrained the simulation should be;
- how much substantiating evidence should be available (for example, there may be partial evidence for the occurrence of certain degradates / metabolites);
- how to supplement the metabolite information with (predicted) toxicological profiles (for example, using QSAR prediction tools).

### 6.2. Mammalian metabolism of carbendazim

In contrast to biodegradation, extensive data on mammalian metabolic pathways for carbendazim are available in the literature (Figure 7). The metabolism of carbendazim has been studied in rats, mice, rabbits, dogs, sheep and cows. The basic biotransformation route involves hydroxylation with the major metabolite, being methyl 5-hydroxybenzimidazol-2-ylcarbamate (5-HBC), and the minor one, being methyl 5,6-dihydroxybenzimidazol-2-

ylcarbamate (5,6-DHCB). The hydroxylated metabolites appear to readily form excretable sulphate and glucuronide conjugates (JMPR, 1999). A small amount of 5,6-HOBC *N*-oxide (mainly as the glucuronide) was also identified. In mammalian organisms detoxification also proceeds through hydrolysis, leading to the formation of 2-aminobenzimidazole (2-AB) as the main metabolite (JMPR, 1999).

A detailed comparison of the metabolites predicted by Meteor 12.0.0 and the OECD QSAR Application Toolbox with the metabolites identified experimentally is provided in Table 9.

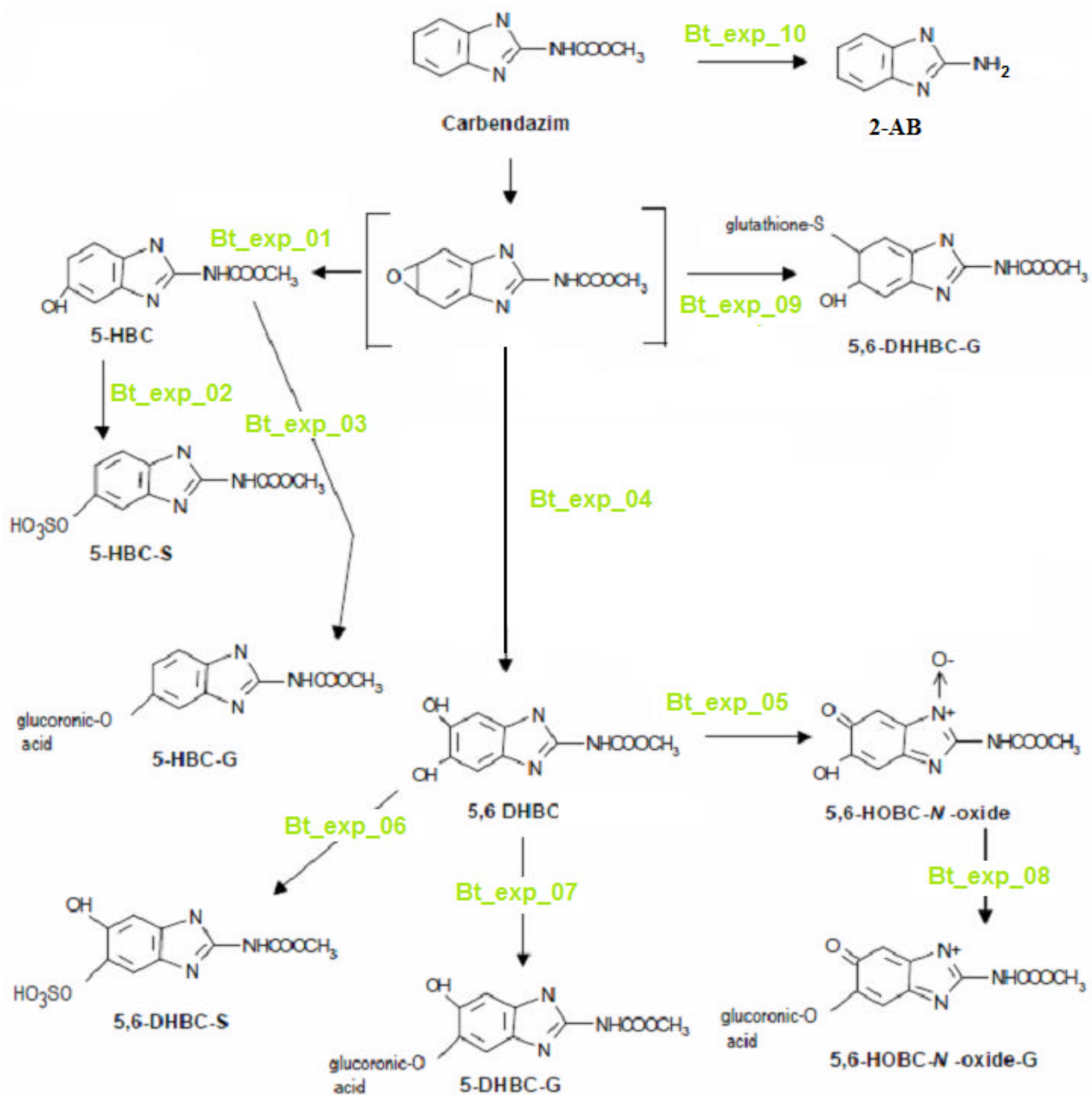
At the first sight, many discrepancies between the observed and simulated biotransformations are noticeable. However, a more in-depth investigation reveals that the differences are not so significant and depend mainly on the constraints defined by the software user. Since Meteor was used to predict the metabolic routes/metabolites formed with at least the "plausible" level of likelihood, it identified only the major product of carbendazim hydroxylation (i.e. 5-HBC) and did not predict the minor metabolite (5,6-DHBC). For the same reason, the metabolite (5-HBC-G) resulting from 5-HCB glucuronidation (Bt\_exp\_03) was predicted, whereas the alternative product (5-HBC-S) resulting from competing sulphation reaction (Bt\_exp\_02) was not predicted, since this reaction did not meet the "plausible" level of likelihood criteria.

For comparative purposes, the mammalian metabolism of carbendazim was simulated with different initial Meteor settings. First, the minimal level of likelihood was changed from "plausible" to "equivocal" and the "global (breadth first search)" option was selected. The simulation resulted in the prediction of 323 different metabolites, resulting not only from the biotransformations identified in the original simulation (i.e. hydrolysis of carbamates, hydroxylation of fused benzenes and glucuronidation of aromatic alcohols), but also from other reactions, e.g. *N*-oxidation at aromatic nitrogen (i.e. Meteor bt #347), being "equivocal" and leading to the formation of metabolite identified experimentally (i.e. 5,6-HOBC-*N*-oxide). In the second alternative simulation, selection of at least the "equivocal" level of likelihood and the "one-at-time (depth first search)" option allowed to identify metabolites such as 5,6-DHBC (formed due to aromatic ring hydroxylation via arene oxides, i.e. Meteor bt #81) and 5,6-DHBC-S (resulting from O-sulphation of aromatic alcohols, i.e. Meteor bt #20). In other words, the results of the metabolic simulation are strictly dependent on the software settings specified by the user, which therefore have to be chosen carefully and in relation to the purpose of investigation.

The mammalian metabolites generated by the OECD QSAR Application Toolbox resulted mainly from two metabolic reactions: hydrolysis and hydroxylation of carbendazim, leading to the formation of 2-AB and 5-HBC, respectively. The metabolites were subsequently transformed into: (i) methanol and 1*H*-benzimidazol-2-ylcarbamic acid, due to further hydrolysis of 5-HBC and (ii) a hydroxylated amine (*N*-hydroxy-1*H*-benzimidazol-2-amine) and some aromatic alcohols (2-AB-5-ol, 2-AB-4-ol, 2-AB-7-ol) due to the hydroxylation of 2-AB.

### 6.3. Parallels between microbial biodegradation and mammalian metabolism

As far as the parallels between microbial biodegradation and mammalian metabolism are concerned, in all cases (i.e. *in silico* and experimental data) hydrolysis and hydroxylation were the most important reactions. All software tools identified the major carbendazim metabolite, 2-aminobenzimidazole (2-AB), which has higher acute toxicity than the parent compound (not acutely toxic). Numerous hydroxylation products, either of the parent molecule or of the formed (intermediate) metabolites, were identified by the three software tools, which is in agreement with experimental data on carbendazim mammalian metabolism.



**Figure 7.** Experimentally documented metabolic pathways of carbendazim in rats (JMPR, 1999)

#### 6.4. General conclusions and recommendations

It is important to be aware that there is no unique set of predictions by most metabolic simulation tools. By changing the model constraints, the user has numerous possibilities to simulate the generation pathways and metabolites, according to the purpose of investigation. For example, only the most probable and significant reactions could be simulated, in which case only a limited amount of major metabolites will be predicted. Alternatively, all imaginable (theoretically possible) biotransformations could be simulated, including those that are highly unlikely to occur under real environmental/biological conditions. Both CRAFT Explorer and Meteor evaluate the probabilities of forming particular metabolites, giving the possibility to expand or restrict the scope of *in silico* simulation, depending on the purpose of the investigation. For example, it might be necessary to identify only the major metabolites, or only the toxicologically relevant metabolites. Both software tools give an insightful interpretation and reasoning of the results and specify the types of competitive reactions considered during simulation. This, in turn, gives the user a rational basis for refining the predictions. Being aware of the reactions neglected by the software (as less probable), the user can apply his/her own knowledge to decide whether the skipped biotransformations should also be taken into account. If the aim of the exercise is to identify toxicologically relevant metabolites, the results of the metabolism simulation can be considered in conjunction with the known or predicted (eco)toxicological properties of the metabolites. To facilitate this process, Meteor provides an integrated interface with the Lhasa Ltd expert system software, Derek for Windows (DfW), which makes predictions for a wide range of ecotoxicological and toxicological effects. A wide range of other prediction tools can also be used to generate toxicological profiles for the metabolites, as reviewed elsewhere (Fuert-Gatnik & Worth, 2010). These profiles can be compared with the toxicological profiles of the parent compound, perhaps in the context of a TTC approach, to assess whether any toxicologically relevant metabolite is associated with a lower TTC than the parent compound. The applicability of these concepts have been explored in a series of EFSA-funded studies (EFSA 2010a, 2010b, 2010c), and further work is ongoing under the auspices of an EFSA Working Group on the Relevance of Pesticide Metabolites to develop practical guidance on how to apply the concepts in regulatory practice. This is an important initiative, which goes beyond the use of computational methods. For example, one issue being considered is what distinguishes a major from a minor metabolite, and how this may depend on the context (whether the metabolite is present in rodents, plants or livestock). Guidance on the interpretation of computer-simulated metabolism is also needed in other areas of regulatory decision making (e.g. industrial chemicals, biocides, cosmetics).

In general, it is concluded that:

- (i) rule-based metabolism simulators open great possibilities to obtain extensive information on the biotransformation routes of chemicals in different species and under different environmental conditions;
- (ii) expertise is needed in the use metabolism simulators, in particular by setting the appropriate scope of simulation;
- (iii) expertise is also needed to interpret the results of the simulation, according to the purpose of the investigation, and taking into account available (corroborating) data
- (iv) further efforts are needed to develop guidance on the interpretation of simulated metabolic pathways and reaction products in the context of environmental and human health risk assessments performed for regulatory purposes. For example, in relation to pesticide risk assessment, there is a need to use methods that provide quantitative or semi-quantitative indicators of the amounts of metabolites formed,



and the timecourses of metabolite generation, in mammals, birds, livestock and plants as well as in the environment. It is also useful to be able to compare the main metabolic pathways in the different species and conditions).

**Table 9.** Comparison of mammalian metabolites predicted by Meteor and the QSAR Application Toolbox with documented experimental data (the major observed routes of biotransformation are marked in bold green; NA – not applicable; \* - microbial metabolites identified by CRAFT)

Experimental Biotransformation No. (Fig. 5) (JMPR, 1999)	Type of Reaction	Observed (Experimental) Metabolite	Predicted by Meteor 12.0.0?	Predicted by the QSAR Application Toolbox?
<b>Bt_exp_01</b>	<b>Hydroxylation of Carbendazim</b>	<b>5-HBC</b>	<b>Plausible</b>	<b>Yes</b>
<b>Bt_exp_02</b>	Sulphation of 5-HCB	5-HBC-S	In original simulation considered as competitive reaction, but less probable than Bt_exp_03. Predicted as "equivocal" in alternative simulation with different settings (and lower minimal level of likelihood).	No
<b>Bt_exp_03</b>	<b>Glucuronidation of 5-HCB</b>	<b>5-HBC-G</b>	<b>Plausible</b>	<b>No</b>
<b>Bt_exp_04</b>	Hydroxylation of Carbendazim	5,6-DHBC*	Predicted as "equivocal" in alternative simulation with different settings (and lower minimal level of likelihood).	No
<b>Bt_exp_05</b>	N-Oxidation of 5,6-DHCB	5,6-HOBC-N-oxide	Predicted as "equivocal" in alternative simulation with different settings (and lower minimal level of likelihood).	No
<b>Bt_exp_06</b>	O-Sulphation of 5,6-DHCB	5,6-DHBC-S	Predicted as "equivocal" in alternative simulation with different settings (and lower minimal level of likelihood).	No
<b>Bt_exp_07</b>	Glucuronidation of 5,6-DHCB	5-DHCB-G	Predicted as "equivocal" in alternative simulation with different settings (and lower minimal level of likelihood).	No
<b>Bt_exp_08</b>	Glucuronidation of 5,6-HOBC-N-oxide	5,6-HOBC-N-oxide-G	Predicted as "equivocal" in alternative simulation with different settings (and lower minimal level of likelihood).	No
<b>Bt_exp_09</b>	Conjugation of with glutathione	5,6-DHHBC-G	Predicted as "equivocal" in alternative simulation with different settings (and lower minimal level of likelihood).	No
<b>Bt_exp_10</b>	<b>Hydrolysis of Carbendazim</b>	<b>2-AB*</b>	<b>Plausible</b>	<b>Yes</b>
<b>Not detected</b>	(Hydrolysis of Carbendazim)	Monomethyl carbonate*	Plausible	NA
<b>Not detected</b>	(Hydrolysis of 5-HBC)	Methanol*	NA	Yes
<b>Not detected</b>	(Hydrolysis of 5-HBC)	1H-benzimidazol-2-ylcarbamic acid*	NA	Yes
<b>Not detected</b>	(Hydroxylation of 2-AB)	N-hydroxy -1H-benzimidazol-2-amine	NA	Yes
<b>Not detected</b>	(Hydroxylation of 2-AB)	2-AB-4-ol; 2-AB-5-ol; 2-AB-7-ol	NA	Yes

## 7. Disclaimer and acknowledgements

Any conclusions and opinions expressed in this document are those of the authors as individual scientists and do not constitute an official position by the JRC or the European Commission.

The authors are grateful to EFSA (Luc Mohimont, Hans Steinkellner, Alf Aagaard, Herman Fontier, Miriam Jacobs) and Lhasa Ltd (Martin Payne, Anthony Long) for their feedback and comments.

## 8. References

Berglof T, Van Dung T, Kylin H & Nilsson I (2002). Carbendazim sorption-desorption in Vietnamese soils. *Chemosphere* 48, 267–273.

Bilinski CA, Sills AM & Stewart GG (1984). Morphological and genetic effects of benomyl on polyploid brewing yeasts: isolation of auxotrophic mutants. *Appl. Environ. Microbiol.* 48, 813–817.

Boethling R, Howard P, Meylan W, Stiteler W, Beauman J & Tirado N (1994). Group contribution method for predicting probability and rate of aerobic biodegradation. *Environ Sci Technol* 28, 459-465.

Boethling RS (1986). Application of molecular topology to quantitative structure-biodegradability relationships. *Environ. Toxicol. Chem* 5, 797-806.

CRAFT (Chemical Reactivity and Fate Tool) Explorer 1.0 (Molecular Networks GmbH, Erlangen, Germany; <http://www.molecular-networks.com/>).

Dimitrov SD, Breton R, Macdonald D, Walker JD & Mekenyan O (2002). Quantitative prediction of biodegradability, metabolite distribution and toxicity of stable metabolites. SAR and QSAR in Environmental Research 13(3-4), 445-455.

Dimitrov SD, Kamenska VB, Walker JD, Windle W, Purdy R, Lewis M & Mekenyan OG (2004). Predicting the biodegradation products of perfluorinated chemicals using CATABOL. SAR and QSAR in Environmental Research 15(1), 69-82.

EC (1991). Council Directive 91/414/EEC of 15 July 1991 concerning the placing of plant protection products on the market. Official Journal of the European Union, L 230/1 of 19.08.1991. Office for Official Publications of the European Communities (OPOCE), Luxembourg.

EFSA (2010a) Applicability of thresholds of toxicological concern in the dietary risk assessment of metabolites, degradation and reaction products of pesticides. Available at: <http://www.efsa.europa.eu/en/scdocs/scdoc/44e.htm>

EFSA (2010b). Impact of metabolic and degradation processes on the toxicological properties of residues of pesticides in food commodities. Available at: <http://www.efsa.europa.eu/en/scdocs/scdoc/49e.htm>

EFSA (2010c). Applicability of QSAR analysis to the evaluation of the toxicological relevance of metabolites and degradates of pesticide active substances for dietary risk assessment. Available at: <http://www.efsa.europa.eu/en/scdocs/scdoc/50e.htm>

EFSA (2010d). Conclusion on the peer review of the pesticide risk assessment of the active substance carbendazim. *EFSA Journal* 8(5), 1598. doi:10.2903/j.efsa.2010.1598. Available at: <http://www.efsa.europa.eu/en/scdocs/scdoc/1598.htm>

Ellis LBM, Roe D & Wackett LP (2006). The University of Minnesota Biocatalysis/Biodegradation Database: The First Decade. *Nucleic Acids Research* 34, D517-D521.

Fuort-Gatnik M & Worth AP (2010). Review of Software Tools for Toxicity Prediction. JRC report EUR 24489 EN. Publications Office of the European Union. Available at: <http://ecb.jrc.ec.europa.eu/qsar/publications/>

Goralczyk K, Strucinski P, Hernik A, Czaja K, Korcz W & Ludwicki JK (2005). Monitoring and official control of pesticide residues in foodstuffs in Poland in 2004. *Roczniki Panstwowego Zakladu Higieny* 56, 307–316. *In Polish.*

Hutson DH (1989). Sulphate ester and glucuronic acid conjugates as intermediates in xenobiotic metabolism. *Intermediary Xenobiotic Metabolism in Animals: Methodology, Mechanisms and Significance*, Hutson DH, Caldwell J & Paulson GD (editors), Taylor and Francis, London, 179-204.

Jaworska J, Dimitrov S, Nikolova N, Masscheleyn P & Mekenyan O (2002). Probabilistic Assessment of Biodegradability Based on Metabolic Pathways: CATABOL System. *SAR and QSAR in Environmental Research* 13(2), 307-323.

JMPR (1999). Pesticide residues in food - 1998 evaluations. Part I - Residues. FAO Plant Production and Protection Paper, 152/1 and 152/2, (two volumes).

Kiso T, Fujita K, Ping X, Tanaka T & Taniguchi M (2004). Screening for microtubule-disrupting antifungal agents by using a mitotic-arrest mutant of *Aspergillus nidulans* and novel action of phenylalanine derivatives accompanying tubulin loss. *Antimicrobial Agents Chemotherapy* 48, 1739–1748.

Magnucka EG, Suzuki Y, Pietr SJ, Kozubek A & Zarnowski R (2007). Action of benzimidazole fungicides on resorcinolic lipid metabolism in rye seedlings depends on thermal and light grow conditions. *Pesticide Biochemistry and Physiology* 88, 219-225.

Mekenyan OG, Dimitrov SD, Pavlov TS & Veith GD (2004). A systemic approach to simulating metabolism in computational toxicology. I. The TIMES heuristic modelling framework. *Current Pharmaceutical Design* 10, 000-000.

Meteor 12.0.0 (Lhasa Ltd., Leeds, United Kingdom; <https://www.lhasalimited.org/index.php/meteor/>).

Mostrag-Szlichtyng A & Worth AP (2010). Review of QSAR Models and Software Tools for predicting Biokinetic Properties. JRC report EUR 24377 EN Publications Office of the European Union. Available at: <http://ecb.jrc.ec.europa.eu/qsar/publications/>

OECD (2009). Guidance Document on the Definition of Residue. Series on Testing and Assessment No. 63, Series on Pesticides No.31. ENV/JM/MONO(2009)30. Organisation for Economic Cooperation and Development, Paris.

OECD QSAR Application Toolbox 2.0 (beta version). <http://www.oecd.org/>.

Okey RW & Stensel H (1996). A QSAR-based biodegradability model – a QSBR. *Water Research* 30(9), 2206-2214.

Pavan M & Worth AP (2008). Review of estimation models for biodegradation. *QSAR and Combinatorial Science* 27, 32-40.

Testa B & Cruciani G (2001). Structure-metabolism relations and the challenge of predicting biotransformation. *Pharmacokinetic Optimization in Drug Research: Biological, Physicochemical and Computational Strategies*, Testa B, van der Waterbeemd H, Folkers G & Guy R (editors), Verlag Helvetica Chimica Acta, Zurich and Wiley-VCH, Weinheim, 65-84.

Tunkel J, Howard PH, Boethling RS, Stiteler W & Loonen H (2000). Predicting ready biodegradability in the Japanese Ministry of International Trade and Industry test. *Environmental Toxicology & Chemistry* 19, 2478-2485.

Vermulen NPE (2003). Prediction of drug metabolism: the case of cytochrome P450 2D6. *Current Topics in Medicinal Chemistry* 3(11), 1227-1239.

European Commission

**EUR 24523 EN – Joint Research Centre – Institute for Health and Consumer Protection**

Title: *In silico* modelling of microbial and human metabolism: a case study with the fungicide carbendazim

Author(s): Aleksandra Mostrag-Szlichtyng and Andrew Worth

Luxembourg: Publications Office of the European Union

2010 – 30 pp. – 21 x 29.7 cm

EUR – Scientific and Technical Research series – ISSN 1018-5593

ISBN 978-92-79-16744-7

doi:10.2788/98567

**Abstract**

A major source of uncertainty when assessing the human health and environmental risks of chemicals is the paucity of experimental information on the metabolic and (bio)degradation pathways of parent compounds and the toxicological properties of their metabolites and (bio)degradation products. Taking into account animal welfare and cost-effectiveness considerations, the only practical means of obtaining the information needed to reduce this uncertainty, is to use alternative (non-animal) methods, such as *in vitro* tests and *in silico* models.

In this report, we explore the usefulness of *in silico* metabolic simulation tools (expert systems) as a means of supporting the regulatory assessment of chemicals. In particular, we investigate the use of selected *in silico* tools to: (i) simulate microbial and mammalian metabolic pathways; (ii) identify potential metabolites resulting from biotransformation; and (iii) gain insights into the mechanistic rationale of simulated metabolic reactions and the likelihood of their occurrence. For illustrative purposes, the microbial and mammalian biotransformation pathways of a case study compound, the fungicide carbendazim, were generated by using the CRAFT Explorer 1.0 (Molecular Networks GmbH) and Meteor 12.0.0 (Lhasa Ltd.) software tools. Additionally, the set of potential metabolites resulting from microbial and mammalian metabolism was predicted with the OECD QSAR Application Toolbox 2.0 (beta version).

Comparison of the *in silico* predictions with existing experimental data on carbendazim metabolism showed the potential usefulness of using software tools for metabolite prediction. However, the results are strongly dependent on the software constraints specified by the user, and require careful interpretation, taking into account the needs of the exercise and the availability of existing information. Further efforts are needed to develop guidance on the use of *in silico* metabolic simulation tools for the purposes of regulatory risk assessments.

### **How to obtain EU publications**

Our priced publications are available from EU Bookshop (<http://bookshop.europa.eu>), where you can place an order with the sales agent of your choice.

The Publications Office has a worldwide network of sales agents. You can obtain their contact details by sending a fax to (352) 29 29-42758.

The mission of the JRC is to provide customer-driven scientific and technical support for the conception, development, implementation and monitoring of EU policies. As a service of the European Commission, the JRC functions as a reference centre of science and technology for the Union. Close to the policy-making process, it serves the common interest of the Member States, while being independent of special interests, whether private or national.

