ANSES Opinion
Request No 2018-SA-0113

The Director General

Maisons-Alfort, 15 January 2019

OPINION
of the French Agency for Food, Environmental and Occupational Health & Safety

on the "assessment of a warning signal regarding the toxicity of succinate dehydrogenase inhibitor (SDHI) fungicides"

ANSES undertakes independent and pluralistic scientific expert assessments.
ANSES's public health mission involves ensuring environmental, occupational and food safety as well as assessing the potential health risks they may entail.
It also contributes to the protection of the health and welfare of animals, the protection of plant health and the evaluation of the nutritional characteristics of food.

It provides the competent authorities with the necessary information concerning these risks as well as the requisite expertise and technical support for drafting legislative and statutory provisions and implementing risk management strategies (Article L.1313-1 of the French Public Health Code).

Its opinions are published on its website.
This opinion is a translation of the original French version. In the event of any discrepancy or ambiguity the French language text dated 15 January 2019 shall prevail.

On 24 May 2018, ANSES issued an internal request to conduct the following expert appraisal: Assessment of a warning signal regarding the toxicity of succinate dehydrogenase inhibitor (SDHI) fungicides.

1. BACKGROUND AND PURPOSE OF THE REQUEST

In an article published on 16 April 2018 in the press, several scientists drew attention to the potential health and environmental risks of using succinate dehydrogenase inhibitor (SDHI) fungicides in agriculture. In this context, ANSES entrusted the analysis of this warning signal to a group of experts.

The objective of this expert appraisal was to determine, based on data from the literature, European assessments of the substances and phytopharmacovigilance data, whether the scientific information and hypotheses mentioned by the authors of the article on the potential health risks of using succinate dehydrogenase inhibitor (SDHI) fungicides in agriculture provided any evidence of exposure or risks that had not been taken into account in the assessments of the fungicidal active substances in question.

2. ORGANISATION OF THE EXPERT APPRAISAL

The expert appraisal was carried out in accordance with French Standard NF X 50-110 "Quality in Expert Appraisals – General Requirements of Competence for Expert Appraisals (May 2003)".

The collective expert appraisal was undertaken by the "SDHI" Emergency Collective Expert Appraisal Group (GECU), supported by the assessment units of the Regulated Products Assessment Department (DEPR), between June and December 2018. The Phytopharmacovigilance and

Observatory of Pesticide Residues Unit (UPO) of the Risk Assessment Department (DER) was also consulted.

The scientists who signed the article were interviewed by the GECU at its meeting on 14 June 2018.

ANSES analyses interests declared by experts before they are appointed and throughout their work in order to prevent risks of conflicts of interest in relation to the points addressed in expert appraisals. The experts' declarations of interests are made public via the ANSES website (www.anses.fr).

3. ANALYSIS AND CONCLUSIONS OF THE GECU

Succinate dehydrogenase inhibitors (SDHIs) are fungicides mainly used on cereals to control major diseases such as septoria leaf spot, net blotch and ramularia leaf spot and as seed treatment for smuts and other fungi not belonging to the Pythiaceae family. They are also used on grapevines, in orchards, and on field crops (other than cereals), vegetable crops and ornamental crops to control major diseases such as *Sclerotinia*, grey mould, *Phoma* and other fungi of this type.

Eleven active substances from this class are currently used in products authorised in France.

Based on the detailed information presented in its report (Annex 2), the GECU notes that:

- It is not possible to draw definitive conclusions regarding all of the issues and hypotheses identified by the researchers issuing the warning,
- Some of these issues and hypotheses involve points common to all phytopharmaceutical active substances and other regulated chemicals: regulatory context not providing for the exclusion of substances on the basis of hazards with the exception of CMR/ED substances, risk management via the application of toxicity thresholds, cumulative exposure, and the predictive nature of ecotoxicology tests for persistent substances. Due to their applicability to all phytopharmaceutical substances, these issues do not constitute an alert specific to the class of SDHIs,
- On the other hand, some other issues and hypotheses are more specific to the class of SDHIs: cross-sensitivity of human and fungal enzymes, introduction of targeted studies in addition to the minimal regulatory studies to consider mitotoxic risks, and the relevance of carcinogenesis studies in rodents for detecting cancers attributable to impaired SDH function.

However, these remaining uncertainties should be read in light of the following information:

- Apparent compliance with good agricultural practices for this class of substances, demonstrated by numerous analyses finding the maximum residue limits (MRLs) to be exceeded only in a few exceptional cases and probably sustained by the need to limit the emergence of fungal resistance,
- Low levels of total dietary exposure in relation to the current toxicological thresholds based on a wide range of tests including carcinogenicity tests in rats,
- Rapid metabolism of these substances leading to low internal doses in relation to external exposure,
- The current state of scientific knowledge regarding the plausibility of a carcinogenic effect of SDH inhibition likely to be reversible and/or limited succinate accumulation,
- The absence, in the current state of the data brought to its attention, of any real signs of a health alert in terms of specific effects observed for environmental organisms,
- The absence, in the current state of the data brought to its attention, of any real signs of a health alert in terms of an increase in the incidence of specific cancers associated with SDH-

deficiency in humans not carrying a mutation (in exposed workers, for example), despite the fact that some of these compounds have been on the market for a long time.

The GECU therefore considers, based on data from the literature, European assessments of the substances and vigilance data, that the scientific information and hypotheses mentioned by the issuers of the warning:

- do not provide any evidence of exposure not taken into account in the assessments of the active substances in question,
- highlight residual uncertainties relating to risks that may not have been taken into account in the assessments of the active substances in question. In the absence of any signs of a health alert, these uncertainties justify the recommendations made in the following paragraph.

In order to resolve certain remaining uncertainties highlighted during the examination of the scientific hypotheses identified by the issuers of the warning, and more broadly to make phytopharmaceutical active substances safer to use, the GECU is issuing the following recommendations, which have been grouped together by theme. These recommendations should be shared at European level, in accordance with the procedures for assessing active substances. Some of them call for the provision of new knowledge, possibly requiring that the safety of use of SDHI active substances be reassessed as knowledge is produced.

To better characterise the hazards associated with SDHI active substances:

- Characterise the inhibition properties of SDHIs and their metabolites and by-products on human enzymes, using appropriate tests and considering combinations of active substances with the same mechanism of action. These inhibition properties should be compared with estimated internal exposure levels for consumers,
- Characterise the inhibition properties of SDHIs and their metabolites and by-products on enzymes of non-target organisms. These inhibition properties should be compared with estimated exposure levels for these organisms,
- Develop the use of detection and characterisation tools for mitotoxic effects that can be used in regulatory assessments.

To better characterise exposure:

- Continue to implement surveillance and control plans providing objective information about actual exposure in the population and in environmental organisms and enabling the data contained in authorisation dossiers to be highlighted,
- Include other SDHI active substances in surveillance and control plans and in future French Total Diet Study work, then update the resulting a posteriori risk assessments,
- Take into account airborne exposure when such data are available, in particular for boscalid which was the only SDHI selected in the expert appraisal on the definition of methods of monitoring pesticides in air.

To better characterise the risks associated with active substances, including SDHIs:

- Test the feasibility of retrospectively and prospectively monitoring changes in the incidence of known diseases involving "SDH" mutations (registries),
- Quantify internal exposure for exposed workers and consumers,
- Carry out work to improve the sensitivity of toxicological and ecotoxicological tests relating to the mechanisms of action of active substances,

- Carry out expert appraisal and research work on cumulative exposure for a given effect, also taking into account common mechanisms of toxic action. In the specific case of SDHIs, this approach should also be applied to combinations of fungicides inhibiting mitochondrial respiration, in particular to document the expected effect in human cells,
- Continue efforts aimed at creating, collecting and interpreting phytopharmacovigilance data in order to detect potential warning signals involving the use of products throughout France,
- Promote the development and use of adverse outcome pathways (AOPs)[1] to consider the combined effects of mixtures[2].

To reinforce the current regulatory schemes:

- Introduce regulatory requirements on the relevance to humans and non-target organisms of the active substances' mechanisms of pesticide action, provided that the target is known and present in humans and/or non-target organisms,
- Consider the possibility of identifying, as with genotoxicity, toxic effects potentially justifying a precautionary approach similar to that described in the regulations applicable to CMR substances.
- Consider using complex ecotoxicological tests simulating natural conditions (cosms) on a more systematic basis,
- Consider the possibility of regularly monitoring non-aqueous matrices (soil in particular) in order to document concentrations of persistent active substances and metabolites and assess the possible cumulative ecotoxic risk after they have been placed on the market,
- Continue the integration of cumulative approaches in regulatory assessment processes.

4. AGENCY CONCLUSIONS AND RECOMMENDATIONS

In light of the GECU's conclusions, the French Agency for Food, Environmental and Occupational Health & Safety considers that the scientific information and hypotheses mentioned by the issuers of the warning do not provide any evidence supporting a health alert that would justify withdrawing the marketing authorisations currently in force in accordance with the national and European regulatory frameworks.

Indeed, considering data from the literature, European assessments of the substances and vigilance data:

- The level of total dietary exposure is low in relation to the current toxicological thresholds, and the MRLs for these active substances are only exceeded in exceptional cases,
- These substances are rapidly metabolised and eliminated,
- With regard to the sources consulted, the experts did not identify any data suggesting an increased incidence of specific cancers associated with SDH-deficiency in humans not carrying a mutation (in exposed workers, for example), despite the fact that some of these SDHI compounds have been on the market for a long time, nor any data suggesting an impact on environmental organisms.

ANSES also endorses the GECU's recommendations aiming to improve knowledge relating to the hazards associated with SDHIs, exposure to these compounds, the risks resulting from this exposure, and the strengthening of the current regulatory schemes, with regard to risk assessment methodologies in particular.

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[1] Sequences of events leading to the occurrence of an in vivo adverse effect, based on the chemical structure of a target chemical or a group of similar chemicals and the molecular initiating event

[2] Souders CL 2nd, Liang X, Wang X, Ector N, Zhao YH, Martyniuk CJ. High-throughput assessment of oxidative respiration in fish embryos: Advancing adverse outcome pathways for mitochondrial dysfunction. Aquat Toxicol. 199 (2018) 162-173.

It should also be noted that ANSES has reported the information presented by the scientists who signed the article at European level, informing the European Commission, EFSA, ECHA and the other Member States. This ANSES Opinion and the report of the Working Group will be sent to these institutions. Moreover, regarding approval or re-approval of active substances in the class of SDHIs, undertaken as part of the procedures defined in Regulation (EC) No 1107/2009, ANSES has already affirmed the need to better take into account mechanisms of SDH inhibition and their potential effects in toxicity assessments of these substances[3].

Thus, these questions will be shared at European level, in accordance with the current assessment procedures for phytopharmaceutical active substances.

Dr Roger Genet

[3] For example, see: Peer review of the pesticide risk assessment of the active substance pydiflumetofen, EFSA Journal (in press)

KEYWORDS

SDHI, fongicide, signal, santé humaine, environnement

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Annex 1

Internal request decision (translation of the original French request document)

2018-SA-0113

Decision No. 2018-05-144

INTERNAL REQUEST

The Director General of the French Agency for Food, Environmental and Occupational Health & Safety (ANSES),

Having regard to the French Public Health Code, and in particular its Article L. 1313-3 giving ANSES the prerogative to issue an internal request on any question with a view to accomplishing its missions,

Has decided the following:

Article 1. The French Agency for Food, Environmental and Occupational Health & Safety is issuing an internal request to undertake an expert appraisal whose characteristics are listed below.

1.1 Themes and objectives of the expert appraisal

The objective is to determine, based on data from the literature, European assessments of the substances, and phytopharmacovigilance data, whether the scientific information and hypotheses mentioned by the authors of an article on the potential health risks of using succinate dehydrogenase inhibitor (SDHI) fungicides in agriculture have provided any evidence of exposure or risks that were not taken into account in the assessments of the fungicidal active substances in question.

1.2 Background of the internal request

In an article published on 16 April 2018 in the press, several scientists drew attention to the potential health risks of using succinate dehydrogenase inhibitor (SDHI) fungicides in agriculture. In this context, ANSES is calling on its experts to consider all the available scientific data on this subject and, in particular, to immediately examine the information mentioned by the scientists issuing the warning. The analysis of this warning signal will be entrusted to a group of experts.

1.3 Questions on which the expert appraisal work will focus

- Based on data from the literature and phytopharmacovigilance data, do the scientific information and hypotheses mentioned by the issuers of the warning provide any evidence of exposure or risks not taken into account in the assessments of the active substances in question?
- If new evidence is found, should it be presented at European level and, if appropriate, should immediate risk management measures be taken for authorised products containing these substances?
- Issue recommendations for follow-up action in response to this warning.

1.4 Estimated duration of the expert appraisal

Three months

Article 2. An opinion will be issued and published by the Agency following completion of the work.

Signed in Maisons-Alfort on 24 May 2018

Dr Roger Genet

Director General

ANNEX 2

"SDHI" GECU report, December 2018

Assessment of a warning signal regarding the toxicity of succinate dehydrogenase inhibitor (SDHI) fungicides

Request No 2018-SA-0113

Collective expert appraisal REPORT

"SDHI GECU"

December 2018

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Keywords

SDHI, fongicide, signal, santé humaine, environnement

SDHI, fungicide, warning signal, human health, environment

Presentation of the participants

PREAMBLE: The expert members of the Expert Committees and Working Groups or designated rapporteurs are all appointed in a personal capacity, *intuitu personae*, and do not represent their parent organisation.

EMERGENCY COLLECTIVE EXPERT APPRAISAL GROUP (GECU)

Chair

Mr Jean-Ulrich MULLOT – Pharmacist (Military Health Service). Specialities: Toxicology, Exposure and Exposure metrology

Members

Ms Marie-France CORIO-COSTET – Research Director (French National Institute for Agronomic Research, INRA). Specialities: Phytopathology, Control methods

Ms Christelle MONTEIL – Teacher-Researcher (University of Rouen). Speciality: Toxicology

Mr Patrick NISSE – Hospital Practitioner (Lille Regional University Hospital). Speciality: Clinical toxicology

HEARINGS WITH EXTERNAL EXPERTS

Scientists who signed the article

Ms Paule BENIT – IR2 Research Engineer at INSERM

Ms Sylvie BORTOLI – Research Engineer – University Unit 1124; "Toxicology, Pharmacology and Cell signalling" team, Paris-Descartes University

Ms Judith FAVIER – Research Director at INSERM

Ms Anne-Paule GIMENEZ-ROQUEPLO – Professor; APHP – INSERM Unit UMR970; "Pheochromocytoma and Paraganglioma" team. Georges Pompidou European Hospital, Paris-Descartes University

Ms Laurence HUC – Research Manager at INRA – INRA-TOXALIM Unit; "Contaminants & Cellular Stress" team, Toulouse-Paul Sabatier University

Mr Manuel SCHIFF – Paediatrician; University Lecturer; Hospital Practitioner at APHP

Ms Malgorzata RAK – Research Manager at CNRS

Mr Pierre RUSTIN – Research Director at CNRS – INSERM Unit UMR1141; "Pathophysiology and Treatment of Mitochondrial Diseases" team. Robert Debré Hospital, Paris-Diderot University

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Acronyms and abbreviations

AAOEL: acute acceptable operator exposure level

ADI: acceptable daily intake

ADME: absorption, distribution, metabolism, excretion

AOEL: acceptable operator exposure level

AOEM: agricultural operator exposure model

ARfD: acute reference dose

AS: active substance

BNVD: French national database of sales of plant protection products

CAG: Cumulative Assessment Group

CMR: Carcinogenic, Mutagenic, Reprotoxic

CP: control programme

DGAL: French Directorate General for Food

DGCCRF: French Directorate General for Competition, Consumer Affairs and Fraud Control

DGS: French Directorate General for Health

ECHA: European Chemicals Agency

ED: endocrine disruptor

EFSA: European Food Safety Authority

GECU: Emergency Collective Expert Appraisal Group

HQ: hazard quotient

HR: high residue

IEDI: international estimated daily intake

IESTI: international estimated short-term intake

iTDS: infant Total Diet Study

ITSAP: Technical and scientific institute for beekeeping and pollination

MA: marketing authorisation

MRL: maximum residue limit

NOAEL: no observed adverse effect level

OECD: Organisation for Economic Co-operation and Development

PBT: Persistent, Bioaccumulative and Toxic

PCC: poison control centre

PEC: predicted environmental concentration

PNEC: predicted no-effect concentration

PPP: plant protection product

RNV3P: National Network for the Monitoring and Prevention of Occupational Diseases

SCoPAFF: Standing Committee on Plants, Animals, Food and Feed

SDH: succinate dehydrogenase

SDHI: succinate dehydrogenase inhibitor

STMR: supervised trials median residue

TDS: Total Diet Study

TMDI: theoretical maximum daily intake

TRV: toxicity reference value

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1. Background, purpose and procedure for carrying out the expert appraisal

1.1 Background

In an article published on 16 April 2018 in the press, several scientists drew attention to the potential health and environmental risks of using succinate dehydrogenase inhibitor (SDHI) fungicides in agriculture. In this context, ANSES entrusted the analysis of this warning signal to a group of experts.

Succinate dehydrogenase inhibitors (SDHIs) are fungicides mainly used on cereals and as seed treatment to control major diseases such as septoria leaf spot, net blotch, ramularia leaf spot, smuts and other fungi not belonging to the Pythiaceae family. They are also used on grapevines, in orchards, and on field crops (other than cereals), vegetable crops and ornamental crops to control major diseases such as *Sclerotinia*, grey mould, *Phoma* and other fungi of this type.

Eleven active substances (ASs) from this class are currently used in products authorised in France.

1.2 Purpose of the request

The objective of this expert appraisal was to determine, based on data from the literature, European assessments of the substances and phytopharmacovigilance data, whether the scientific information and hypotheses mentioned by the authors of the article on the potential health and environmental risks of using succinate dehydrogenase inhibitor (SDHI) fungicides in agriculture provided any evidence of exposure or risks that had not been taken into account in the assessments of the fungicidal active substances in question (see Annex 1).

1.3 Procedure: means implemented and organisation

ANSES entrusted the examination of this formal request to the "SDHI" Emergency Collective Expert Appraisal Group (GECU).

This work was therefore conducted by a group of experts with complementary skills.

The expert appraisal was carried out in accordance with French Standard NF X 50-110 "Quality in Expert Appraisals – General Requirements of Competence for Expert Appraisals (May 2003)".

The GECU took into account European assessments of active substances in the class of SDHIs, data from the literature and pharmacovigilance data brought to its attention.

1.4 Prevention of risks of conflicts of interest

ANSES analyses interests declared by experts before they are appointed and throughout their work in order to prevent risks of conflicts of interest in relation to the points addressed in expert appraisals.

The experts' declarations of interests are made public via the ANSES website (www.anses.fr).

2. Regulations on the marketing of plant protection products

2.1 Safety assessment of an active substance

Regulation (EC) No 1107/2009 of 21 October 2009 lays down, for all Member States, conditions for the approval of active substances and the marketing of plant protection products.

Active substances can only be included in plant protection products where "*it has been demonstrated that they present a clear benefit for plant production and they are not expected to have any harmful effect on human or animal health or any unacceptable effects on the environment. In order to achieve the same level of protection in all Member States, the decision on acceptability or non-acceptability of such substances should be taken at Community level on the basis of harmonised criteria".* Indeed, active substances are assessed at European Union (EU) level. The decision to approve or re-approve an active substance rests with the European Commission (EC) after a scientific assessment by the European Food Safety Authority (EFSA).

Plant protection products are preparations containing one or more active substances, responsible for the product's properties, as well as substances called co-formulants which give the product a form suitable for its application. Before a plant protection product can be placed on the market, it must demonstrate that it presents *"a clear benefit for plant production"* and does not *"have any harmful effect on human or animal health, including that of vulnerable groups, or any unacceptable effects on the environment".* The assessment of plant protection products may be national or zonal (there are three zones in Europe, with France belonging to the South zone). In France, marketing authorisations (MAs) are issued by ANSES.

Regulation (EC) No 1107/2009 provides guidelines for the assessment of hazards and risks. It is supplemented by implementing regulations, including Regulation (EU) No 546/2011 which deals with risk acceptability criteria. These documents indicate the technical data that must be contained in application dossiers, as well as the methods to be implemented to obtain them. They also specify, where appropriate, threshold values above which the risk should be considered unacceptable or additional tests to require to refine the assessment. These regulations, supplemented by various guidance documents, ensure that dossiers are assessed in the same way from one Member State to the next.

Dossiers concerning active substances must enable their intrinsic properties to be characterised and therefore the hazards they pose for humans and the environment. They must include the following:

- Overview and physico-chemical properties
- Validated methods of analysis in plants, water, soil, air and foodstuffs of animal origin likely to contain residues of the substance
- Data on the mechanism of action
- Toxicity and metabolism studies in mammals, undertaken in accordance with the EC or OECD (Organisation for Economic Co-operation and Development) guidelines and in compliance with GLP (Good Laboratory Practices)
- Studies on metabolism and residues in plants (and in foodstuffs of animal origin where appropriate)
- Studies on the fate and behaviour of the active substance in soil, groundwater, surface water and air
- Ecotoxicity studies undertaken with the active substance and its major degradation products.

The assessment of plant protection products focuses on:

- the quality and efficacy of the products;
- the risks that their use may pose for applicators during treatment, farm workers handling the treated plants and any persons passing close by during application, as well as people living close by;
- risks to consumers;
- risks to the environment and wildlife.

An MA application is submitted for one or more specific uses, a use being defined for the crop treated, the target pest (parasites, weeds, etc.), the quantity of product used per hectare, the period, the method of application and the frequency of use. These results are then examined in light of the decision-making criteria (called "uniform principles") set out in Regulation (EU) No 546/2011, enabling a conclusion to be drawn as to the acceptability of the assessed risks. For assessments conducted in the context of MA renewal, data from various surveillance networks (presence in water, observation of exposure or poisoning cases in humans, etc.) are incorporated into the assessment.

Active substances are managed and assessed at EU level. The "Phytopharmaceuticals – Pesticides Legislation" section of the Standing Committee on Plants, Animals, Food and Feed (SCoPAFF), composed of Member State representatives, decides on the approval of active substances.

EFSA is responsible for assessing the substances by calling on the expertise of Member States.

When a company wishes to seek approval for a new active substance under Regulation (EC) No 1107/2009, it must put together a dossier and submit it to the Member State of its choice. This Member State, appointed as the "rapporteur", then examines the dossier and prepares a draft assessment report, which is sent to EFSA. EFSA forwards this draft report to the other Member States, collects their comments and organises discussions between experts from these Member States. EFSA's final assessment report is sent for review to the European Commission, which proposes an approval or non-approval decision. ANSES is in charge of reviewing the dossier when France is the "rapporteur Member State" for an active substance.

For the assessment of plant protection products, a distribution of the associated workload across geographical zones has been put into place. MA applicants must submit a dossier for a complete assessment in one of the Member States in the zone. Other Member States in the zone in which an MA is also requested will then rely on the assessment report prepared by that Member State. There is also a procedure of mutual recognition between Member States from different zones, in which the assessment conducted by the Reference Member State serves as a basis simplifying the review of applications.

2.2 Assessment of hazards for human health

The assessment of a phytopharmaceutical active substance includes the identification and characterisation of hazards related to its intrinsic properties and an assessment of the associated risks, taking the claimed uses into account.

In terms of human health, *in vivo* and *in vitro* experimental studies are undertaken using validated protocols, in accordance with good laboratory practices and Regulation (EU) No 283/2013, in order to determine the characteristics described below. The tests required for the assessment of risks are listed in Annex 2.

Absorption, distribution, metabolism and excretion (ADME) studies in mammals

An *in vivo* ADME study, usually conducted in rats, enables the oral absorption, distribution, metabolism and excretion of the active substance to be characterised. The data generated are used to establish toxicokinetic constants as well as the metabolic profiles.

An *in vitro* metabolism study comparing metabolism in human material (microsome or intact cellular system) with the metabolism in the species tested in toxicity studies is also required in order to assess similarities and differences in metabolic profiles. It is necessary to generate additional data when a metabolite is detected *in vitro* in human material and not in the tested animal species.

Acute toxicity and local tolerance

Toxicity studies after a single administration by the oral route, dermal route or inhalation enable lethal doses/concentrations of the substance to be established.

Studies on skin and eye irritation and dermal sensitisation enable local tolerance to be determined.

An *in vitro* phototoxicity study is also required for substances absorbing electromagnetic radiation having a wavelength between 290 and 700 nm.

Short-term toxicity

Toxicity studies after repeated administration for 90 days, conducted in several species (rats, dogs and mice), enable general and specific toxic effects to be identified as well as their reversibility and potential target organs for short-term exposure. For each study, a no observed adverse effect level (NOAEL) must be established, and relationships between doses and adverse effects must be characterised.

Genotoxicity

As part of a sequential approach, *in vitro* and *in vivo* genotoxicity tests exploring the mutagenic, clastogenic and aneugenic potential of an active substance are required to ensure the absence of *in vivo* genotoxic potential.

Long-term toxicity and carcinogenicity

Toxicity studies following repeated administration for two years in rats and 18 months in mice are required to identify harmful effects and establish a NOAEL for lifetime exposure. These tests should also enable the detection of potential carcinogenic effects resulting from prolonged exposure to the active substance. If a carcinogenic response is detected, it is necessary to determine species, sex and organ specificity and explore the underlying mode of action and its relevance to humans.

Reprotoxicity

Multi-generational studies should make it possible to document impaired reproductive function or capacity in males and females, identify direct and indirect effects on several generations, determine the most susceptible exposure windows and establish NOAELs for parental toxicity, fertility and offspring development.

The developmental toxicity studies conducted in various species (rats and rabbits) aim to identify direct and indirect effects on embryonic and foetal development, including teratogenic effects, and establish NOAELs for maternal toxicity and development.

Neurotoxicity

Specific neurotoxicity studies are required if the active substance is a structural analogue of a neurotoxic compound, if neurotoxic effects were observed in the general toxicity studies or if its mode of pesticide action relies on a neurotoxic mechanism.

Other toxicological studies

Metabolite toxicity

If metabolites found in foodstuffs or groundwater differ from those found in the animals tested in toxicological studies, additional tests are necessary to identify and characterise the hazards associated with metabolites relevant to human health.

Additional studies with the active substance

In order to clarify any issues raised in the aforementioned studies, it may be necessary to carry out additional individually designed tests.

Endocrine-disrupting effects

If an active substance has effects suggesting endocrine disruption, additional information or specific studies are required to explain the mechanism of action and explore the associated adverse effects.

Medical data

When available, human data (medical surveillance of personnel from the production facility, clinical cases, epidemiological studies) must be taken into account.

In addition to the tests required under the regulations, a systematic review of the published literature must be carried out in order to collect and analyse the available scientific information on the toxicity of the active substance and its relevant metabolites.

Identification of hazards for human health

This corpus of studies and information enables human health hazards to be identified.

A harmonised classification of the active substance is thus established by comparing the observed adverse effects with the criteria for the various classes of human health hazards from Regulation (EC) No 1272/2008[1].

In addition, certain approval criteria for phytopharmaceutical active substances rely on their classification in accordance with Regulation (EC) No 1107/2009 (Article 4 and Annex II). For example, a substance classified as a cat.1A or cat.1B CMR substance[2] cannot be approved.

2.3 Human health risk assessment

This corpus of studies and information also enables the risks to human health to be characterised via the establishment of toxicity reference values (TRVs). For each TRV established for the duration of exposure, route of exposure and population in question, the source study used is that in which the lowest NOAEL was observed. Uncertainty factors are applied to this critical dose to take into account, among other things, differences between species and individuals and variations between the experimental conditions and actual conditions of exposure in the population.

1 Regulation (EC) No 1272/2008 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 16 December 2008 on classification, labelling and packaging of substances and mixtures, amending and repealing Directives 67/548/EEC and 1999/45/EC, and amending Regulation (EC) No 1907/2006.

2 Classified CMR substances: substances inducing carcinogenic, mutagenic or reprotoxic effects.

For phytopharmaceutical substances, four TRVs are established: an acceptable daily intake (ADI)[3] and an acute reference dose (ARfD)[4] dedicated to the assessment of risks for dietary exposure, as well as an acceptable operator exposure level (AOEL)[5] and an acute acceptable operator exposure level (AAOEL)[6] dedicated to the assessment of risks for non-dietary (i.e. occupational or environmental) exposure.

Assessment of risks via non-dietary exposure

Systemic exposure is estimated for operators[7], workers[8], bystanders[9] and residents[10], via the use of a suitable calculation model (i.e. the AOEM model developed by EFSA[11]), taking into account the claimed uses and the proposed conditions of use.

Health risks are then characterised by comparing the estimated exposure levels with the AOEL for sub-chronic exposure or with the AAOEL for acute exposure.

If relevant, this estimation should focus on the cumulative and synergistic effects resulting from exposure to more than one active substance and to toxicologically relevant compounds contained in the product.

Assessment of risks to consumers[12]

In the EU, the *a priori* assessment, placing on the market and post-approval (*a posteriori*) monitoring of plant protection products and pesticide residues in food are harmonised (Regulation (EC) No 396/2005, Regulation (EC) No 1107/2009). This regulatory framework helps ensure that the residual levels of active substances (ASs) measured in foods do not pose any risks to consumers (EFSA, 2014). Regulation (EC) No 396/2005 defines the term "pesticide residues" as "residues, including active substances, metabolites and/or breakdown or reaction products of active substances currently or formerly used in plant protection products [as defined by Directive 91/414/EEC repealed by Regulation (EC) No 1107/2009], including in particular those which may arise as a result of use in plant protection [Regulation (EC) No 1107/2009], in veterinary medicine [Regulation (EC) No 37/2010] and as a biocide [Directive 2008/98/EC]".

Maximum residue limits (MRLs) of active substances must be set for each foodstuff according to Regulation (EC) No 396/2005. These limits correspond to the highest concentrations of pesticide residues legally authorised in each foodstuff for each phytosanitary active substance. They help ensure compliance with good agricultural practices on the one hand and limit consumer exposure to

3 The acceptable daily intake (ADI) of a chemical is the estimate of the amount of an active substance in food or drinking water that can be ingested daily over a lifetime without appreciable health risk to the consumer on the basis of all the known facts at the time of the evaluation. It is expressed in milligrams of the chemical per kilogram of body weight (WHO, 1997).

4 The acute reference dose (ARfD) of a chemical is the estimate of the amount of a substance in food or drinking water that can be ingested over a short period of time, usually during one meal or one day, without appreciable health risk to the consumer on the basis of all the known facts at the time of the evaluation. It is expressed in milligrams of the chemical per kilogram of body weight (WHO, 1997).

5 The acceptable operator exposure level (AOEL) is the maximum amount of active substance to which the operator may be exposed daily without any adverse health effect. It is expressed in milligrams of the chemical per kilogram of body weight.

6 The acute acceptable operator exposure level (AAOEL) is the maximum amount of active substance to which the operator may be exposed over the course of a day without any adverse health effect. It is expressed in milligrams of the chemical per kilogram of body weight.

7 Operators are farming professionals who carry out activities linked to the application of pesticides, i.e. mixing and loading of pesticides into machinery, as well as operating, cleaning, emptying or repairing such equipment.

8 Workers are people who, as part of their job, enter an area previously treated with pesticides or who handle crops treated with pesticides.

9 Bystanders are people who may be in or next to an area treated with pesticides and who take no protective measures.

10 Residents are people living, working or attending school near an area where pesticides are used and who take no protective measures, such as wearing special clothing, to reduce exposure.

11 AOEM: Agricultural Operator Exposure Model https://efsa.onlinelibrary.wiley.com/doi/10.2903/j.efsa.2014.3874

12 Sarda X., Merlo M., Nougadère A. Résidus de pesticides dans les aliments. In: Camel V., Riviere G., Le Bizec B. Risques chimiques liés aux aliments. Paris: Lavoisier, 2018, third part, chapter 19, p. 345. ISBN: 978-2-7430-2388-1.

pesticide residues on the other hand. They are not directly associated with risks but can be used for assessing them. Thus, exceeding an MRL does not necessarily imply a risk to consumers.

MRLs apply throughout the EU, in order to guarantee the safety of consumers while ensuring the free circulation and marketing of foodstuffs in Europe, whether from Member States or from third countries (import tolerances). Regulation (EC) No 396/2005 clearly defines the respective roles of the Member States, EFSA, the European Commission and the European Parliament in the establishment of MRLs and specifies harmonised MRLs[13], the foodstuffs or group of foodstuffs[14] for which an MRL should be set, and a list of low-risk substances not requiring an MRL[15].

A database[16], managed by the European Commission, centralises the MRLs in force for the relevant active substances and foodstuffs in addition to the corresponding toxicity reference values.

At the time the products concerned are placed on the market, Regulation (EC) No 1107/2009 states that it is necessary to demonstrate that each product does not pose risks to consumers and complies with the set MRLs and the conclusions of the European assessment of the AS.

When a plant protection product is applied to a crop, the AS it contains will persist for some time on the plants, be degraded into metabolites and/or be washed off by rainwater. The consumer risk assessment consists in identifying and quantifying all compounds (parent compounds and metabolites) likely to be found in foods following crop treatment. This analysis should take into account the treated plants as well as foodstuffs of animal origin (if these plants are intended for animal feed) and processed foods. The assessment should also take into account the behaviour of the compounds in soil in order to consider their possible uptake by plants subsequently cultivated on the same plots as the treated crops. This assessment helps ensure that the products placed on the market do not pose any risks to consumers and enables MRLs to be set.

The studies required for the *a priori* consumer risk assessment are listed in Regulation (EC) No 283/2013; they must comply with the guidelines prepared by the OECD member countries. Guidelines on food safety are grouped together in Section 5 (Series 500) (OECD, 2013a). The steps of this assessment are described below.

Hazard characterisation: qualitative aspect

Relevant residues for consumer safety (parent compound and/or metabolites) contained respectively in plants, foodstuffs of animal origin, processed products and rotation crops, are defined on the basis of metabolism studies on the active substance in plants and farm animals, supplemented by studies on the fate of active substances during industrial and domestic processing and during crop rotations. These studies are taken into account to define the residue for the risk assessment on the one hand, i.e. to identify all of the toxicologically relevant compounds present in significant quantities, and to define the residue for control and surveillance on the other hand, i.e. to identify any toxicologically relevant, abundant and easily quantifiable compounds.

Estimation of residue accumulation in foodstuffs: quantitative aspect

All of the compounds included in the definition of residue are screened for in foodstuffs. To do so, tests undertaken in accordance with good agricultural practices, measuring residue levels in plants, as well as animal feed studies and studies quantifying residues in industrially processed products and rotation crops lead to the proposal of MRLs or transfer factors for unprocessed and processed products.

13 Annex II to Regulation (EC) No 396/2005

14 Annex I to Regulation (EC) No 396/2005

15 Annex IV to Regulation (EC) No 396/2005

16 http://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/public/?event=homepage&language=EN

These studies also enable, for a given active substance and foodstuff, a supervised trials median residue (STMR) value and the "highest residue" (HR) value to be estimated for the implementation of the risk assessment.

Characterisation of risks to consumers

For the *a priori* estimation of a chronic risk, the proposed MRLs are assigned to each of the corresponding foodstuffs contained in the European consumption models, to define a maximum concentration per foodstuff and an ingested dose depending on the diet. The sum of the daily ingested doses is used to estimate consumer exposure, i.e. calculate the theoretical maximum daily intake (TMDI), which must be lower than the ADI in order for the risk to be considered as acceptable. If the TMDI is higher than the ADI, it is necessary to refine the risk by calculating the international estimated daily intake (IEDI) with the STMRs available for each foodstuff and compare it once again to the ADI.

Similarly, for the calculation of an acute risk, the international estimated short-term intake (IESTI) is calculated based on the HR values for each foodstuff and consumption model data; it must be lower than the ARfD in order for the risk to be considered as acceptable. There is no possible refinement for acute risk.

Post-marketing risk assessment

Regulation (EC) No 396/2005 provides for the yearly implementation of a coordinated Community control programme (Article 29), as well as national monitoring programmes dedicated to the assessment of risks (Article 30). In practice, most of the pesticide residues analysed as part of the monitoring programmes are residues of phytopharmaceutical ASs but they can also include residues of veterinary antiparasitics and/or biocides.

These monitoring programmes aim to verify compliance with current legislation (ensuring that the MRLs are not exceeded, among other things) and assess consumer risks in order to guide:

– risk managers in the establishment of their monitoring programmes and the implementation of preventive and corrective measures;

– risk assessors in the orientation of research and expert appraisal work, especially that involving metrology, exposure assessment and toxicology.

Every year, for the EU, EFSA summarises the analysis results produced as part of the coordinated Community programme and the monitoring programmes of the various Member States and undertakes an *a posteriori* assessment of dietary exposure and food risks. This work gives rise to the publication of a complete annual report, available on the EFSA website[17].

In France, the monitoring of exposure and risks related to pesticide residues in food fits into this European regulatory framework. It is the responsibility of ANSES and relies on two complementary tools:

– an "overall" quantitative risk assessment method, based on four chronic and acute indicators updated on a regular basis to take into account the results of the most recent national monitoring programmes as well as the MRLs;

– multi-year Total Diet Studies (TDSs). These studies focusing on the analysis of levels in foods as consumed (on the consumer's plate) are more realistic but do not enable acute consumer risk to be assessed. They are undertaken every seven to 10 years or so.

17 https://www.efsa.europa.eu/en/efsajournal/pub/5348

The French monitoring programmes covering the marketing of foodstuffs are implemented by the Directorate General for Competition, Consumer Affairs and Fraud Control (DGCCRF) for fruits and vegetables, cereals and infant food, the Directorate General for Food (DGAL) for foodstuffs of animal origin, and the Directorate General for Health (DGS) for water intended for human consumption (Directive 98/83/EC).

With regard to food consumption data, these two approaches are based on the results of the French Individual and National Food Consumption Survey (INCA 2) undertaken by ANSES in 2006-2007.

This *a posteriori* national assessment has the advantage of providing good coverage of the national diet and including a large number of substances in order to estimate the exposure of French consumers as precisely and objectively as possible.

2.4 Assessment of ecotoxicological hazards and risks

The assessment of risks to the environment and environmental organisms is broken down into three steps:

- Step 1: assessment of hazards for environmental organisms (ecotoxicity)
- Step 2: assessment of exposure for environmental organisms (predicted environmental concentrations (PECs))
- Step 3: risk assessment

A risk assessment is conducted for organisms present in various environments likely to be impacted by the use of the active substance: organisms in soil (macro- and micro-organisms), birds and mammals, bees and other non-target arthropods, non-target plants and aquatic organisms.

Step 1: assessment of hazards for environmental organisms (ecotoxicity)

The objective of this step is to determine the toxicity to organisms of an active substance and a product. Among other things, it enables the various effects of an active substance, its degradation products (metabolites) or a product on the various classes of organisms to be identified.

The assessment of toxicity to organisms, or ecotoxicity, is based on species meant to be representative of biological diversity in natural environments. For each major group of organisms (e.g. fish, crustaceans, insects and plants for aquatic environments), the toxicity of substances and products is estimated by undertaking standardised tests with farmed organisms whose susceptibility has been established. These tests are conducted to determine the acute (resulting in the death of the organism after a short exposure period) and chronic (preventing the organism from developing and/or reproducing after a long exposure period) toxicity of the tested substance. They also serve as the basis for the environmental classification of active substances and products, appearing on the packaging of products on the market, in accordance with Regulation (EC) No 1272/2008. The tests required for the assessment of ecotoxicological risks are listed in Annex 2.

Since not all living species can be tested, one or more model organisms are chosen to represent the others. For example, to estimate the toxicity of substances or products for coldwater fish, the laboratory organism is rainbow trout. Safety factors are applied to the results obtained in order to minimise uncertainty related to differences in susceptibility between organisms of the same species and organisms of different species. These factors enable a risk acceptability threshold to be determined for all organisms.

To supplement these laboratory ecotoxicology tests, trials simulating natural conditions can also be undertaken in order to assess effects on ecosystems. For example, for macro-organisms in soil, field trials are conducted with the monitoring of earthworm populations after products are applied. For aquatic organisms, the systems in which these trials are undertaken are called microcosms (micro-ecosystems) or mesocosms (meso-ecosystems) depending on their size.

Step 2: assessment of exposure for environmental organisms (predicted environmental concentrations (PECs))

This step is intended to determine the quantities of active substance and possible degradation products to which the environment may be exposed during the use of a product. The objective is to estimate the quantities of substances and degradation products that may be present, for example, in soil and surface water. For substances likely to persist in the environment, exposure calculations must take potential environmental accumulation into account.

These quantities are estimated using models that take into consideration the various possible pathways to the different compartments of the environment. For example, for bodies of surface water (rivers, ditches, ponds), the following are considered: spray drift, artificial drainage (since some agricultural plots are equipped with underground drains to evacuate excess water) and runoff for surface water.

These models enable all pathways to be taken into account, considering:

- the formation of degradation products in the various compartments (soil, water and sediment),
- properties specific to each substance and its degradation products in soil and water (solubility, retention capacity and rate of degradation in soil and sediment),
- the properties of the natural environment (soil type, climate, crop).

In the case of a marketing application for a plant protection product, modelling is performed for each requested use, i.e. for a crop, an application dose per hectare, a number of applications per year and a period of application during the year. This enables predicted concentrations in soil, surface water, sediment and groundwater to be estimated.

Step 3: assessment of risks

The assessment of risks for various classes of organisms combines hazard (ecotoxicity) and environmental concentrations (exposure).

The concentrations to which various organisms may be exposed (PECs) are compared with the results of acute and chronic toxicity tests. Toxicity/PEC ratios are thus calculated.

If these ratios are above the risk acceptability threshold (which corresponds to the safety factor), the risk is acceptable under the defined conditions of use. This means that exposure is lower than a concentration considered as having no effect for aquatic organisms. If these ratios are below the risk acceptability threshold, the risk is not acceptable under these conditions. In some cases, to reduce the exposure of organisms, it is possible to introduce mitigation measures that will need to be implemented when using the product.

The assessment of risks to environmental organisms within the regulatory framework for the marketing of products is undertaken based on toxicity data specific to each active substance and each product. Thus, in the event of a product containing several active substances, a potential combined effect of the substances may be observed in the tests.

After MAs have been issued, phytopharmacovigilance data may be taken into account to ensure, among other things, that the use of products in accordance with good agricultural practices does not generate unexpected effects on the environment or on non-target environmental organisms.

3. List of SDHI substances and authorised uses

According to FRAC[18], 18 active substances assessed in Europe belong to the class of SDHIs. This is a fairly recent class of active substances, most of which (excluding boscalid, carboxin and flutolanil) have been approved since 2013. Twelve of these substances are currently approved in Europe. Four SDHI substances have not been authorised in the EU since 2002: benodanil, fenfuram, mepronil and oxycarboxin. Furametpyr and thifluzamide are not included in the European Commission's EU database of ASs authorised for use as plant protection products (PPPs).

In France, 11 of the active substances approved in the EU are contained in 46 reference products for around 100 parallel trade permits and one generic product, representing a total of 133 uses (see Annex 3). Penflufen has been approved in Europe since 2014 but there are no authorised products in France containing this active substance. The data given in the following table correspond to the current or previous regulatory scheme. For certain products authorised within previous regulatory frameworks, provisional authorisations may have been issued pending the active substance's approval under the new system: that is why this table includes some dates of first PPP authorisation that are earlier than that of the active substance.

Table 1: List of SDHI active substances approved in the EU

SDHI list (source: FRAC)	**Main types of use**	**AS's most recent approval date**	**Date of first authorisation of a PPP containing the AS**
Benzovindiflupyr	Foliar application: cereals	03/2016	11/2016
Bixafen	Foliar application: cereals	10/2013	08/2011
Boscalid	Foliar application: cereals, grapevines, orchards, cruciferous oilseed crops, sunflower, vegetables	08/2008	06/2005
Carboxin	Seed treatment	06/2011	12/1968
Fluopyram	Foliar application: cereals, orchards, vegetable crops, oilseed crops, bananas	02/2014	10/2013
Flutolanil	Seed treatment: potatoes	09/2009	06/1992
Fluxapyroxad	Seed treatment and foliar application: cereals, orchards, vegetables	01/2013	10/2011
Isofetamid	Seed treatment and foliar application: grapevines, orchards, strawberries, cruciferous oilseed crops	09/2016	08/2018
Isopyrazam	Foliar application: ornamental crops	04/2013	12/2017
Penthiopyrad	Foliar application: cereals and tomatoes	05/2014	11/2014
Sedaxane	Seed treatment: cereals and maize	02/2014	07/2011

18 Fungicide Resistance Action Committee

4. Sales, use and vigilance data involving SDHIs

4.1 Sales and use data

Sales and use data are collected via the French national database of sales of plant protection products by distributors (BNVD[19]) as well as the "cropping practices" surveys of the Ministry of Agriculture.

Boscalid is the best-selling substance in the class of SDHIs. It currently ranks no. 49 for sales of substances used in PPPs, with almost 250 tonnes sold by professionals in 2016. However, its annual sales tonnage is on the decline, since 600 tonnes were sold in 2009 (when it ranked no. 22 for sales).

In terms of cropping practices, boscalid is applied at least once to almost 80% of the rapeseed-growing area (2014 data), 51% of the carrot-growing area (2013 data), around 30% of the growing area for strawberries, lettuce (2013 data) and apples (2012 data), and around 20% of the growing area for grapevines (2014 data), melons and leeks (2013 data). Note that for soft wheat and barley, almost 30% of the growing area was treated with boscalid at least once in 2011 versus around 10% during the 2014 survey. Conversely, 11% of grapevines were treated with boscalid at least once in 2011 versus 22% in 2014.

Sales tonnages for carboxin and flutolanil are lower and, as with boscalid, are decreasing:

- Carboxin: 43.2 tonnes (ranked no. 121) versus 76.4 tonnes in 2012
- Flutolanil: 4.6 tonnes (ranked no. 218) versus 13.1 tonnes in 2009

For these substances, no treated areas have been observed in the surveys of cropping practices. This is because these surveys do not take into account seed-treatment use, which is the main authorised use for these substances.

For more recently authorised substances (since 2013), sales are on the rise but have not reached the level of boscalid. Their sales figures are as follows, in decreasing order:

- Fluxapyroxad: 145 tonnes in 2016 (ranked no. 69) versus 113 tonnes in 2013, with 38% of the growing area for soft wheat treated at least once in 2014 and 24% of the barley-growing area in the same year
- Bixafen: 90 tonnes in 2016 (ranked no. 86) versus 82 tonnes in 2013, with 38% of the barley-growing area treated at least once in 2014 and 22% of the growing area for soft wheat in the same year
- Fluopyram: 60 tonnes in 2016 (ranked no. 106) versus 10 tonnes in 2014
- Sedaxane: 20 tonnes in 2016 (ranked no. 154) versus 13 tonnes in 2013
- Benzovindiflupyr: 656 kg in 2016 (ranked no. 281) versus 0 in 2015
- Penthiopyrad: 432 kg in 2016 (ranked no. 291) versus 0.4 kg in 2015

For these last four substances, no treated areas have been observed in the surveys of cropping practices. The most recent data available from these surveys on field crops date from 2014[20].

For isofetamid and isopyrazam, no sales data are recorded in the BNVD.

19 Seed-treatment sales have only been included in the BNVD since 2012.

20 A survey of practices for "field crops" is currently under way but the data are not yet available.

Figure 1: Change in SDHI sales between 2008 and 2017 in % per year in France

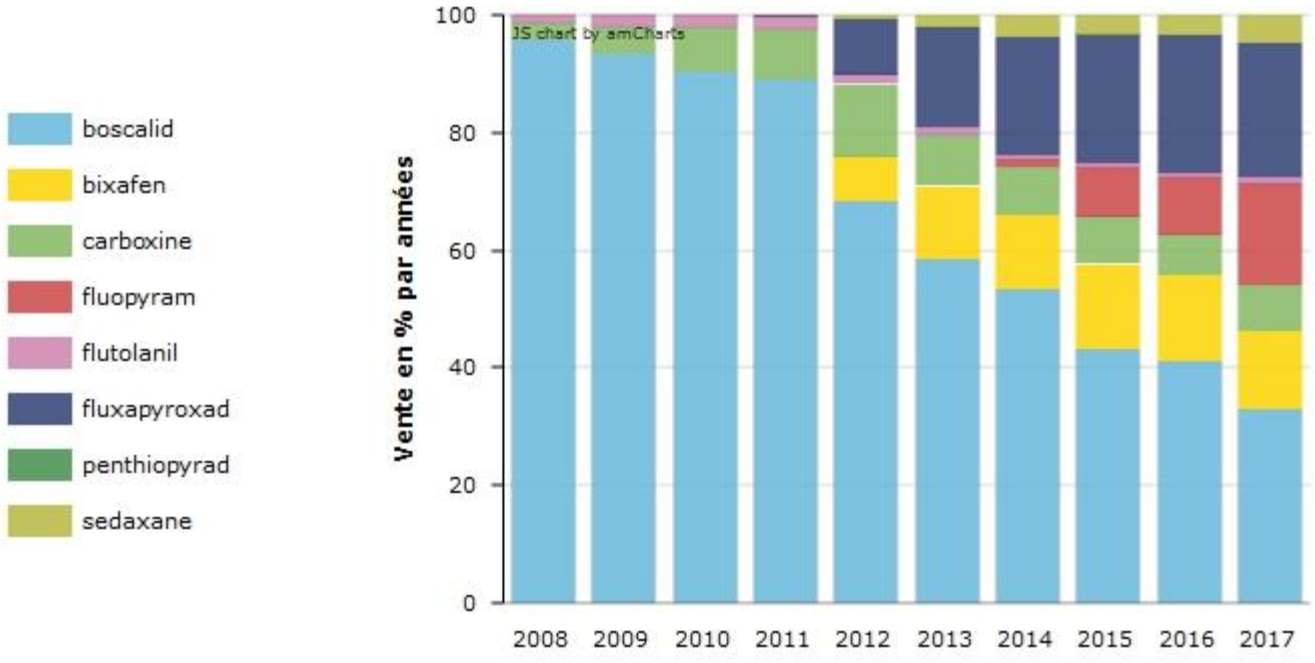
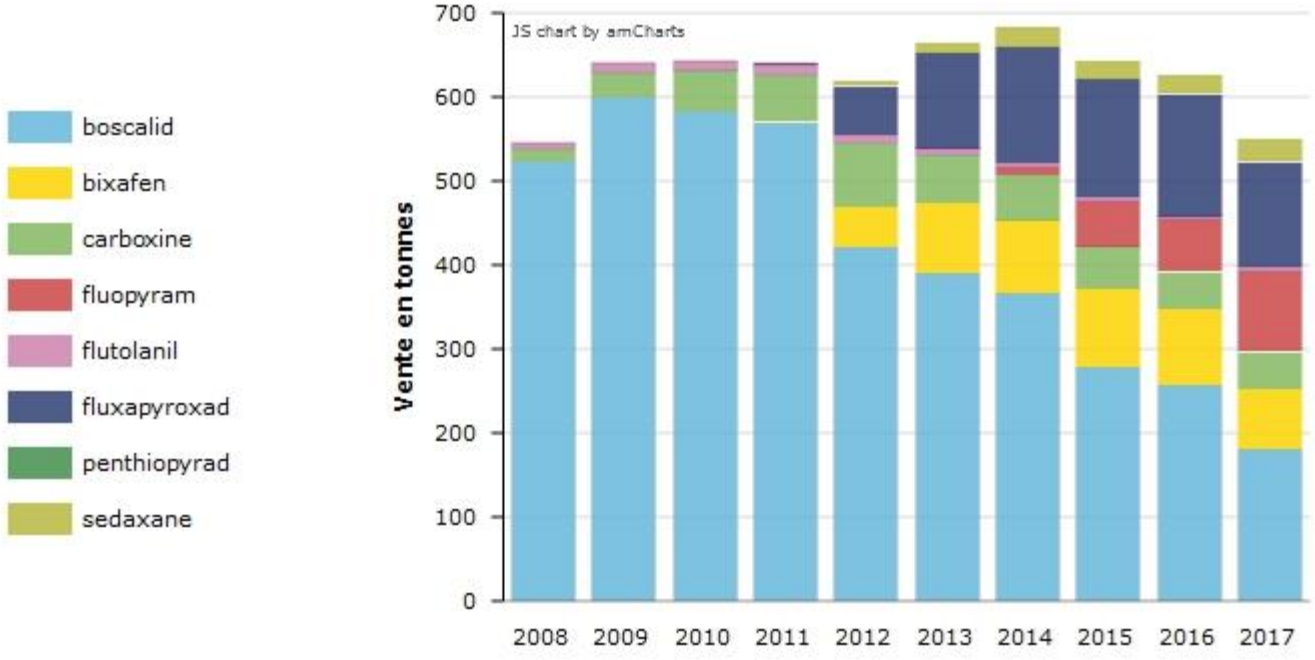


Figure 2: Change in SDHI sales between 2008 and 2017 in tonnage per year in France



These figures show a decline in sales since 2008 for boscalid and an increase for substances approved more recently, for a total volume of 500 to 700 tonnes per year, suggesting a shift from boscalid to the use of more recent SDHIs.

4.2 Phytopharmacovigilance data

The data discussed below have been taken from the phytopharmacovigilance system. The methodology used to collect these data is available in the explanatory note on the summary sheets of monitoring and vigilance data by active substance (*Notice explicative des fiches de synthèse des données de surveillance et de vigilance par substance active*, ANSES, November 2017)[21].

4.2.1 Foodstuffs[22]

The data relate to France and the three most recent available years, from 2013 to 2016.

For boscalid: this substance is screened for in almost 5000 analyses carried out every year with foods collected during distribution. The quantification rates vary from 4.4% to 8.7% depending on the year. Between 110 and 150 types of foods are analysed. No exceeding of the MRLs has been observed.

This substance is also directly analysed in foods during production, with between 1200 and 2700 analyses carried out every year, corresponding to 69 to 77 types of foods. Regarding foods tested directly during production, the quantification rates are higher (between 9.2% and 12.7%) but have not resulted in the MRLs being exceeded in recent years.

In the second Total Diet Study (TDS2), 611 analyses were undertaken for boscalid, with a quantification rate of 3.1%[23] and no exceeding of the MRLs. In the infant Total Diet Study (iTDS), 305 analyses were carried out, with a quantification rate of 1.6% and no exceeding of the MRLs.

For flutolanil: this substance is screened for in almost 5000 analyses carried out every year with foods collected during distribution. The quantification rate is 0.02%. Between 109 and 139 types of foods are analysed. No exceeding of the MRLs has been observed (except in one carrot in 2015).

This substance is also directly analysed in foods during production, with between 1200 and 2600 analyses carried out every year, corresponding to 65 to 70 types of foods. The quantification rates are below 0.7% and have not resulted in the MRLs being exceeded in recent years.

In the TDS2, 313 analyses were carried out for flutolanil, with no observed quantification. In the iTDS, 296 analyses were carried out, with no observed quantification.

For carboxin: this substance is screened for in 2200 to 4500 analyses carried out every year with foods collected during distribution, without any quantification. Between 109 and 119 types of foods are analysed.

This substance is also directly analysed in foods during production, with between 800 and 1400 analyses carried out every year, corresponding to 50 to 61 types of foods. Only one quantification has been observed and has not resulted in the MRLs being exceeded in recent years.

In the TDS2, 211 analyses were carried out for carboxin, with no observed quantification. In the iTDS, 135 analyses were carried out, with no observed quantification.

Other substances were not screened for in the TDS2 and iTDS given their more recent approval (bixafen, fluxapyroxad, fluopyram, sedaxane, penthiopyrad, benzovindiflupyr). They are screened for in surveillance and control plans, to varying degrees:

21 https://www.anses.fr/fr/system/files/Notice_explicative_Fiches_Phytopharmacovigilance.pdf

22 Sources: surveillance and control plans implemented by the Ministries of Agriculture and Consumer Affairs, and ANSES Total Diet Studies: TDS2 (ANSES, 2011, French Total Diet Study 2, Volume 2: Pesticide residues, additives, acrylamide, PAHs, June 2011, Scientific ed., 401 pages) and iTDS (ANSES, 2016, infant Total Diet Study, Volume 2, Part 4: Results relating to pesticide residues, collective expert appraisal report, September 2016, Scientific ed., 378 pages)

23 The pooling of foods in the TDS studies does not enable the observed concentrations to be compared with the MRLs for each individual sample

- bixafen and fluopyram are screened for during distribution (between 2200 and 4800 analyses per year) and production (between 400 and 1800 analyses per year), with a quantification rate below 2.5%. One case of the MRL being exceeded in kiwis was observed for fluopyram.
- fluxapyroxad is screened for in foods during distribution (between 2200 and 4500 analyses per year) and production (300 to 960 analyses per year), without any quantification.
- penthiopyrad and benzovindiflupyr are only screened for in foods during production and in 2016 (respectively 551 and 187 analyses per year), without any quantification.
- there are no data available on screening for sedaxane for the 2013-2016 period.

4.2.2 Surface water[24]

The data below relate to the three most recent available years, from 2012 to 2015.

Regarding the presence of substances in the SDHI class in surface water, boscalid stands out from the other substances. For metropolitan France, boscalid is screened for at 52% to 62% of the measurement points in the surveillance network, i.e. a total of 11,000 to 16,000 analyses per year. The quantification rates range from 11% to 18% without exceeding the PNEC. A factor of more than 600 is observed between the aquatic PNEC and the highest contamination means.

Flutolanil and carboxin are also screened for in surface water at 29% to 67% of the measurement points, i.e. 8000 to 15,000 analysis results per year, with low quantification rates below 0.2% not exceeding the PNEC. Factors of more than 600 and 1200 are observed between the PNEC and the highest contamination means, respectively for carboxin and flutolanil.

There are no available data in the French overseas departments and regions (DROMs) for these substances.

Fluxapyroxad and bixafen are seldom screened for (less than 2% of the measurement points in the surveillance network, corresponding to 300 analyses per year at most), with only one quantification for fluxapyroxad in 2015 that did not exceed the PNEC. These substances are associated with lower PNECs than that of boscalid, respectively by a factor of 4 for fluxapyroxad and 27 for bixafen.

For fluopyram, sedaxane, penthiopyrad and benzovindiflupyr, there are no surveillance data for surface water.

4.2.3 Water intended for human consumption[25] and groundwater[26]

Non-conformities are analysed with regard to exceeding the current regulatory quality threshold of 0.1 $\mu g \cdot L^{-1}$ in water intended for human consumption.

In water intended for human consumption, for the 2007-2016 period:

- For boscalid: more than 5000 analyses are carried out each year, with a quantification rate below 0.6% and four non-conformities since 2014.
- For flutolanil and carboxin, around 1500 analyses are carried out each year, with one quantification and no non-conformities since 2014.
- Fluxapyroxad and bixafen are seldom screened for (30 analyses per substance in 2016), without any quantification.
- The other substances are not screened for (fluopyram, sedaxane, penthiopyrad, benzovindiflupyr).

24 Source: French Ministry of the Environment

25 Source: health monitoring of water intended for human consumption of the French Ministry of Health

26 Source: French Ministry of the Environment

For groundwater, the available results have been analysed for the 2014-2016 period.

- More than 15,000 analysis results are available for boscalid, with a quantification rate of 5%. The non-conformity rate is 0.4%.
- For flutolanil, more than 8000 analysis results are available, with a quantification rate of 0.1% and no observed non-conformities.
- Carboxin and bixafen are screened for (more than 7000 and 4400 analysis results respectively), with only one quantification for bixafen.
- The other substances are not screened for (fluxapyroxad, fluopyram, sedaxane, penthiopyrad, benzovindiflupyr).

4.2.4 Food risk assessment for consumers[27]

Exposure to residues of plant protection products is calculated based on data on body weights, food consumption and residue levels in foods collected via surveillance and control plans (ANSES, 2014)[28] or Total Diet Studies (TDSs).

For boscalid, according to the available data, no exceeding of the chronic or acute toxicity reference values has been observed. According to a worst-case scenario, chronic exposure represents no more than 1.2% of the ADI, with 60% to 95% coverage of the diet.

For flutolanil, according to the available data, no exceeding of the chronic or acute toxicity reference values has been observed. According to a worst-case scenario, chronic exposure represents no more than 0.2% of the ADI, with 65% to 92% coverage of the diet. For the TDS2, since the available analyses do not provide adequate coverage of the entire diet, the risks to consumers cannot be estimated.

For carboxin, since the available analyses do not provide adequate coverage of the entire diet, the risks to consumers cannot be estimated.

Food risks, assessed in MA applications, have not been assessed using this complementary approach based on the measurements available for other substances (fluxapyroxad, bixafen, fluopyram, sedaxane, penthiopyrad, benzovindiflupyr) due to their more recent introduction on the French market and the time required to implement surveillance and control plans.

4.2.5 Ambient air[29]

Only boscalid was screened for in the last three available years (2013 to 2015), with a quantification rate below 1% estimated based on 250 to 320 analyses per year.

As part of the expert appraisal work on the definition of methods of monitoring pesticides in air[30], SDHI substances were prioritised. Only boscalid was identified as a priority for the national monitoring of pesticides in air[31].

27 Source: ANSES

28 ANSES, 2014. ANSES Opinion on the updated food risk indicators for pesticide residues. Response to Request No 2013-SA-0138, p. 26 + Annexes (in French)

29 Sources: AASQA and FédéAtmo

30 ANSES (2017). ANSES Opinion and Report on the proposed arrangements for national surveillance of pesticides in ambient air. Maisons-Alfort. 306 pages (in French).

31 Boscalid was identified as a priority using these two approaches due to its use and potential for occurrence in the air compartment

4.2.6 Cases of animal poisoning (wildlife and domestic animals)[32]

According to the available data (ONCFS data on 31/12/2013), no acute effects on wildlife were identified. Moreover, despite potential exposure to boscalid and flutolanil, these substances were not detected in contamination measurements for granivorous birds or their unhatched eggs in farmlands[33,34].

Regarding domestic animals (CAPAE-Ouest data on 31/12/2017), the CAPAE-Ouest poison control centre has received few calls involving SDHI fungicides (one for boscalid, seven for carboxin, one for fluopyram, two for sedaxane) and these have mostly been related to the ingestion of treated seeds (by dogs in particular). That is why they are more common in the autumn planting period. Poisoning was not deemed "Probable" for any of the calls received, usually due to:

- the unspecific toxicity of these active materials,
- the substances generally being used in combination,
- unknown ingested doses,
- the ingestion of seeds, which may also have played a role in the disorders observed.

4.2.7 Massive acute mortality[35] and contamination of bee matrices[36]

4.2.7.1 Massive acute mortality

Between 2012 and 2016, 660 reports of bee mortality were received as part of the nationwide surveillance of massive acute mortality and diseases, classified as Category 1 health hazards in bees. In the 27 investigations concluding there had been poisoning with one or more active substances, no deaths were attributed to SDHIs.

4.2.7.2 Contamination of bee matrices

According to the available data (ITSAP – on 22/06/2018), boscalid and fluopyram have been found in bee matrices, primarily in trap pollen.

Table 2: Concentrations of boscalid in bee matrices (expressed in mg/kg)

Results	**Trap pollen**	**Bee bread**	**Honey**
Number of analyses	1007	356	109
LOQ	0.02	0.02	0.02
Occurrence of detection	161	2	1
Frequency of detection (%)	16	0.6	0.9
Occurrence of quantification	101	1	1
Frequency of quantification (%)	10	0.3	0.9

32 Sources: ONCFS and GIS-Toxinelle

33 Bro E, Millot F., Decors A., Devillers J. Quantification of potential exposure of gray partridge (Perdix perdix) to pesticide active substances in farmlands. Sci Tot Env (2015) 521-522: 315-25

34 Bro E., Devillers J., Millot F., Decors A. Residues of plant protection products in grey partridge eggs in French cereal ecosystems. Env Sci and Poll Research (2016) 23: 9559-73

35 Source: DGAL

36 Source: Technical and scientific institute for beekeeping and pollination (ITSAP – Bee Institute)

Results	Trap pollen	Bee bread	Honey
Mean concentration	0.169	-	-
Maximum concentration	3.2	0.026	0.04
Median concentration	0.057		
P5	0.024		
P95	0.544		

Table 3: Concentrations of fluopyram in bee matrices (expressed in mg/kg)

Results	Trap pollen
Number of analyses	1007
LOQ	0.01
Occurrence of detection	50
Frequency of detection (%)	5
Occurrence of quantification	24
Frequency of quantification (%)	2.4
Maximum concentration	0.278

The contamination of samples of trap pollen collected by bees stretches out over the entire colony-monitoring season, i.e. from April to September for boscalid. For fluopyram, contamination runs from April to June. The two substances are mainly found in apiaries around which there is a higher concentration of grapevines.

4.2.8 Human data

4.2.8.1 Human biomonitoring[37]

There are no human biomonitoring data for these substances[38] as part of the national programme.

There are no data on occupational exposure in agriculture. Indeed, these substances are not part of the chemical classes analysed in the Pestimat crop-exposure matrices or in the Pestexpo programme (dermal and respiratory exposure under real-life conditions). At this point in time, there are no plans to add them to the work programmes of these two projects.

The aim of the POPEYE project (*Exposition aux pesticides dans la cohorte mères-enfants Elfe et Issues de grossesse*) was to describe exposure to pesticides in the ELFE national mothers-and-children cohort and its determinants and assess the possible impact of exposure during pregnancy on its outcome. This project relied on the ELFE cohort of around 18,300 mother-child pairs recruited in maternity departments in metropolitan France in 2011. As part of this project, boscalid was quantified in the hair of a sub-sample of pregnant women: of the 311 tested samples,

37 Source: French Public Health Agency – François Baclesse Centre

38 These substances were not identified as priorities when establishing the list of compounds to be included in the national biomonitoring programme.

195 (63%) were above the limit of detection and the median concentration measured was 0.55 pg/mg of hair[39]. In this study, these concentrations were deemed representative of internal exposure due to hair-washing before the assays. The concentrations of boscalid were different between the two represented regions (Aquitaine and Champagne-Ardenne according to the administrative boundaries of the time), which appeared in line with the respective farming practices of these two regions.

The concentrations of pesticides in hair were considered in light of the risk of cryptorchidism and anthropomorphic parameters (birth weight and height) within the studied population. Boscalid was included in these analyses but no significant association with this substance was found[40]. Analyses are also in progress in connection with dietary exposure to pesticides, estimated via consumption data for these women and contamination data from the TDS2, but the results are not yet available.

4.2.8.2 Toxicovigilance data

4.2.8.2.1 RNV3P data

The National Network for Monitoring and Prevention of Occupational Diseases (RNV3P) is a network of occupational health professionals that includes the 30 occupational disease clinics (CCPPs) in metropolitan France and the overseas territories. Its aim is to record data from CCPP consultations in a national database (demographic patient data, diseases, exposure, sector of activity, profession, etc.). Following an investigation, expert physicians at the CCPPs establish a potential connection between the exposure situation(s) and the disease that motivated the consultation (the causal link is recorded in the database).

The RN3PV database does not contain any cases of paragangliomas or pheochromocytomas – diseases which, according to the researchers interviewed by ANSES's Emergency Collective Expert Appraisal Group, can be associated with SDHI exposure – in workers exposed to pesticides.

4.2.8.2.2 Poison control centre (PCC) data

A query of the national database of PCC cases between 1 January 1999 and 30 June 2018 inclusive resulted in the selection of 30 symptomatic acute exposure cases where causality was non-null and there was no co-exposure, including 29 cases arising from unintentional circumstances and one from an intentional circumstance (suicide attempt). No cases of chronic exposure were recorded in this database; in each of the 30 acute cases, only one person was exposed.

Occupational exposure accounted for all of the cases of unintentional exposure and no serious cases were reported in this series.

Twenty-five of these 29 cases involved a PPP containing an SDHI fungicide combined with one or two other fungicides not belonging to this class.

With exposure by inhalation, the reported symptoms included respiratory disorders (cough, irritation of the upper airways, coloured sputum, sensation of chest tightness), ENT disorders (oropharyngeal pain, epistaxis), digestive disorders (vomiting, diarrhoea, abdominal pain, dysgeusia) and neurological and neuromuscular disorders (headaches, dizziness, myalgia); asthenia, discomfort, an episode of bradycardia and an isolated case of hyperthermia were also observed. No cases of bronchial spasms or episodes of respiratory distress were reported.

With splashing in eyes, ocular pain (six cases) and conjunctivitis/conjunctival erythema (six cases) were mainly found. No cases of keratitis were reported in this series.

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39 Beranger, R., E. M. Hardy, C. Dexet, L. Guldner, C. Zaros, A. Nougadere, M. A. Metten, C. Chevrier and B. M. R. Appenzeller (2018). Multiple pesticide analysis in hair samples of pregnant French women: Results from the ELFE national birth cohort. Environ Int 120: 43-53

40 Beranger, R., E. M. Hardy, A.-C. Binter, M.-A. Charles, B. M. R. Appenzeller and C. Chevrier (2017). Association Between Hair-Concentrations of Pesticides During Pregnancy and Birth Weight: A Multipollutant Approach from the Elfe Birth Cohort. ISEE, Sydney, Australia.

With dermal exposure, the symptoms most commonly reported were skin irritation, localised erythema and pruritus; paresthesia with tingling and dizziness was described in one case.

With the one case of digestive exposure, ingestion was uncertain and the reported symptoms were dizziness accompanied by blurred vision with spontaneous regression.

It should be noted that an antabuse syndrome (vomiting, dizziness, bradycardia, abdominal pain and pale skin) occurred following exposure to a product containing carboxin combined with thiram. This set of symptoms was linked to thiram due to alcohol intake during exposure and before the occurrence of the symptoms.

In these 25 cases, the SDHIs involved were carboxin (six cases), fluxapyroxad (six cases), boscalid (five cases), flutolanil (three cases), bixafen (three cases) and sedaxane (two cases). They were combined with other fungicides:

- imidazoles or triazoles: prothioconazole (three cases), tebuconazole (one case), epoxiconazole (nine cases), metconazole (one case), difenoconazole (two cases);
- strobilurin: pyraclostrobin (five cases);
- phenylpyrrole: fludioxonil (two cases);
- dithiocarbamates: mancozeb (three cases) and thiram (six cases).

Moreover, four of the 29 cases involved a boscalid (two cases) or flutolanil (two cases) PPP not containing any other fungicidal substances in the formula. The circumstances were related to a professional activity in all of the four cases.

There were benign symptoms in all of the cases and recovery without any sequelae.

Three cases involved respiratory exposure, with headaches (two cases), coughing (one case), nausea (one case), vomiting (one case) and abdominal pain (one case).

The fourth and final case involved dermal exposure: paresthesia with tingling of the lips was reported.

In conclusion, over the 1999-2018 study period (18.5 years), 29 cases of unintentional acute occupational exposure to a PPP containing an SDHI were reported. For PPPs containing an SDHI combined with active substances belonging to other classes of fungicides, irritative symptoms were dominant and seemed primarily related to the presence either of these substances or of co-formulants. For PPPs only containing an SDHI, the reported symptoms were unspecific; moreover, no additional information can be provided regarding the acute human toxicity of this group of active substances due to the very small study population (four cases).

4.2.8.2.3 Data from the Phyt'Attitude network

During the period from 1997 to 31 August 2018, the Phyt'Attitude[41] network of the French Central Fund for the Agricultural Mutual Insurance Scheme (MSA) recorded 12 reports involving a commercial product containing an SDHI active substance alone or combined with one or more other active substances, with no co-exposure to other plant protection products, where causality was at least plausible[42].

These 12 reports involved eight commercial products and nine active substances including three belonging to the class of SDHIs (carboxin, flutolanil and boscalid) (Table 4).

41 The Phyt'Attitude network, created in 1991 by the MSA (Agricultural Mutual Insurance Scheme), is made up of occupational physicians, prevention officers and expert toxicologists who identify, analyse and validate information about incidents occurring during the use of plant protection products. The operating principle of Phyt'Attitude relies on the voluntary reporting of adverse effects by users of these products, which means that the reports are not exhaustive or representative of the entire agricultural world; furthermore, the situations of multiple exposure faced by farm workers (multiple plant protection products, biocidal products, exhaust gases, paints, solvents, etc.) constitute a limitation to the interpretation of the data and in particular their extrapolation to a given active substance. Despite these limitations, this network's strength lies in the provision of accurate information, based on feedback from the field and combining medical, technical and contextual data.

42 A causality score is assigned to each product/disorder-symptom pair: the overall causality score for the dossier corresponds to the highest assigned causality score. Causality scores range from I0 to I4: excluded, unlikely, plausible, likely, very likely.

Only one of the eight products involved only contained an SDHI active substance, and it gave rise to one report.

Table 4: Number of reports and active substances

carboxin	N	flutolanil	N	boscalid	N
+ thiram	4	not in association	1	+ kresoxim-methyl	2
+ thiram + anthraquinone	1	+ mancozeb	2	+ pyraclostrobin	1
+ prochloraz + anthraquinone	1				
Total	6		3		3

It should be noted that of all the products behind these reports, only three are currently authorised: one containing flutolanil only, one containing boscalid and kresoxim-methyl, and another containing boscalid and pyraclostrobin. The other products involved, containing carboxin combined with thiram, anthraquinone, mancozeb or prochloraz, have been withdrawn from the market, some a very long time ago (highlighted in Table 4).

All "disorder-product" causality scores combined and regardless of the product (SDHI alone or combined with one or more other substances), 33 signs and symptoms were reported. Of them, 28 were considered as having plausible or likely causality. There were:

- hepato-digestive disorders such as diarrhoea, digestive pain (poorly localised), epigastric pain, oropharyngeal pain and nausea (eight reports);
- neurosensory-eye symptoms: unspecified vision problems, conjunctivitis/conjunctival erythema, watery eyes (seven reports);
- neurosensory-nose symptoms: irritation of the upper airways, rhinitis/rhinorrhoea (six reports);
- skin reactions, such as contact dermatitis and pruritus (three reports);
- neurological and neuromuscular symptoms: headaches (two reports);
- respiratory symptoms such as coughing (two reports).

With regard to authorised products, exposure to the product containing flutolanil alone resulted in severe epigastric pain in one subject with a history of gastric ulcer, during application by manually dusting powder onto a field vegetable crop for three hours (this corresponds to misuse). A causality score of "plausible" was assigned.

Exposure to the product combining boscalid and kresoxim-methyl was responsible for mucocutaneous irritation phenomena (watery eyes, rhinorrhoea, pruritus on exposed areas accompanied by nausea) in two employees of the same company when bringing plants back into a greenhouse, 12 hours after application. A causality score of "likely" was assigned.

The product combining boscalid and pyraclostrobin was involved in the occurrence of oral paresthesia and a burning sensation in the throat with an irritative cough accompanied by slight dyspnea as well as transient dizziness and diarrhoea in one employee, on the 10th consecutive day of treatment of garlic cloves. The person was working outdoors was preparing the solution and loading the machine used for clove treatment, and was monitoring the spraying of the solution. He was wearing a chemical protective suit (type 5-6), nitrile gloves, eyeglasses and respiratory protection (filtration device with a P3 dust filter and an A2 gas-vapour filter). It should be noted that the personal protective equipment was not worn systematically or continuously, especially during the hottest periods of the day. A causality score of "plausible" was assigned.

In light of these reports, since the two SDHI substances flutolanil and boscalid have not been classified for human toxicity, the mucocutaneous irritation reactions observed in the three cases may have been related to the presence of another active substance or irritating co-formulants in the product.

5. Hypotheses relating to SDHI active substances identified by the researchers issuing the warning

The documents shared by the researchers issuing the warning and identified by the GECU, as well as the hearing held in June 2018, served to highlight the main functions of SDH in humans and its role in diseases on the one hand and to identify the various hypotheses constituting the warning according to its issuers, on the other hand.

5.1 Pathophysiology of human SDH

SDH, also called succinate-ubiquinone oxidoreductase (EC 1.3.5.1) or mitochondrial respiratory chain complex II, is an enzyme that contains four subunits (SDHA, SDHB, SDHC, SDHD) encoded by exclusively nuclear deoxyribonucleic acid sequences. SDHA, a flavoprotein, and SDHB, an Fe-S protein, are two subunits located in the mitochondrial matrix whereas SDHC and SDHD are embedded in the inner membrane. Together they have two active sites, one for the oxidation of succinate to fumarate and one for the reduction of ubiquinone to ubiquinol[43,44]: these coupling functions between oxidative phosphorylation and the Krebs cycle are essential for the proper functioning of mitochondrial respiration and therefore play a central role in all energy-consuming cellular functions. Subunits A and B of this heteroprotein are better conserved across species than subunits C and D.

Despite the central role of the enzymes of the respiratory chain, it has been demonstrated since the 1990s that cellular life remains compatible despite Krebs cycle disruptions induced by mutations in the encoding genes for these enzymes. For those more specifically addressed in this expert appraisal, several inherited and fewer sporadic mutations in the various subunits of human SDH have been identified since 1995 and associated with different diseases discussed in several literature reviews[45,46,47,48,49,50,51]. These have mainly been cancerous (pituitary adenoma and paraganglioma/pheochromocytoma, renal cell carcinoma, gastrointestinal tumours), neurological (encephalopathy, leukodystrophy, Leigh syndrome) and cardiac (cardiomyopathy) diseases sometimes occurring during childhood. Similarly, epigenetic alterations in key sequences of these genes can modify the protein expression of SDH subunits and be associated with certain cancers[52].

Mutation-related diseases are currently considered as rare and are often detected in the form of clustered family cases[53]. The tumours are usually benign but there is potential for malignancy, as tumours associated with SDHB gene mutations are more likely to become malignant. The transmission of hereditary pheochromocytoma-paraganglioma is autosomal dominant (with

43 J.-J. Brière, J. Favier, V. El Ghouzzi, F. Djouadi, P. Bénit, A.-P. Gimenez, P. Rustin. Review - Succinate dehydrogenase deficiency in human. CMLS, Cell. Mol. Life Sci. 62 (2005) 2317–2324

44 C. Bardella, P.J. Pollard, I. Tomlinson. Review - SDH mutations in cancer. Biochimica et Biophysica Acta 1807 (2011) 1432–1443

45 P. Rustin, T. Bourgeron, B. Parfait, D. Chretien ,A. Munnich, A.Rötig. Inborn errors of the Krebs cycle: a group of unusual mitochondrial diseases in human. Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease 1361 (1997) 185-197

46 P. Rustin, A. Rörig. Inborn errors of complex II – Unusual human mitochondrial diseases. Biochimica et Biophysica Acta (BBA) - Bioenergetics 1553 (2002) 117-122

47 P. Bénit et al. A new threat identified in the use of SDHIs pesticides targeting the mitochondrial succinate dehydrogenase enzyme. DOI: dx.doi.org/10.1101/289058

48 Bourgeron T. et al. Mutation of a nuclear succinate dehydrogenase gene results in mitochondrial respiratory chain deficiency. Nat Genet. 11 (1995) 144-9.

49 Parfait B, Chretien D, Rötig A, Marsac C, Munnich A, Rustin P. Compound heterozygous mutations in the flavoprotein gene of the respiratory chain complex II in a patient with Leigh syndrome. Hum Genet.106 (2000) 236-43.

50 Levitas A. et al. Familial neonatal isolated cardiomyopathy caused by a mutation in the flavoprotein subunit of succinate dehydrogenase. Eur J Hum Genet. 18 (2010) 1160-5.

51 Rao JU. et al. Genotype-specific differences in the tumor metabolite profile of pheochromocytoma and paraganglioma using untargeted and targeted metabolomics. J Clin Endocrinol Metab. 100 (2015) 2014-2138.

52 Richter S. et al. Epigenetic Mutation of the Succinate Dehydrogenase C Promoter in a Patient With Two Paragangliomas. The Journal of Clinical Endocrinology & Metabolism *101 (2016)* 359–363

53 Hereditary forms of pheochromocytoma (PHEO)-paraganglioma (PGL) account for 30% of cases. The prevalence of PHEOs is around 1/500,000 and that of PGLs around 1/1,000,000 (source: Orphanet)

incomplete penetrance), but the SDHD and SDHAF2 genes are subject to maternal genomic imprinting and expressed when the mutation is inherited from the father. Penetrance depends on the gene, age and tumour site. The mechanisms involved in carcinogenesis as a result of highly SDH-deficient cells are partly understood and at least partially related to succinate accumulation whose role in tumorigenesis and tumour progression was recently demonstrated[54]. To cite just one piece of evidence supporting the role of succinate accumulation in tumorigenesis, the observed overexpression of hypoxia-inducible transcription factors (HIFs) and some of their target genes appears to enable tissue and cellular adaptation to hypoxia and give tumour clones the ability to selectively proliferate. It appears that in SDH-deficient cells, accumulated succinate behaves as an oncometabolite whose role in epigenetic cellular regulation contributes to the promotion of the tumour phenotype.

5.2 Hypotheses constituting the warning, according to its issuers

According to the researchers who issued the warning, current knowledge on the role and functioning of SDH and on the health consequences of loss of SDH activity enable the following hypotheses to be put forth in the context of presence on the national and international markets of phytopharmaceutical active substances (SDHIs) inhibiting the SDH activity of the target pests:

- Inadequacy of the "conventional" toxicological tests required for the marketing of an active substance, for the specific case of the toxicity assessment of SDHIs, for the following reasons:
    - Mitotoxicity should be considered as a separate hazard, which itself can provide grounds for management measures (similarly to genotoxicity, for example), without there being a need for toxicity tests. According to the issuers of the warning, the hazardous nature of this mechanism of action has already led to other mitotoxic active substances, including cyanide salts and rotenone, being withdrawn from the market in the past,
    - The inadequacy of conventional genotoxicity tests for detecting carcinogenic effects associated with SDH deficiency,
    - The inadequacy of murine models, used during chronic toxicity tests, for detecting the toxicity and/or carcinogenicity of SDHIs.
- The existence or high probability of health effects in humans consuming contaminated foods, comparable to those identified in patients carrying SDH mutations (essentially tumours). The hypothesis of health effects resulting from the use of these active substances by professionals (non-dietary exposure) was also mentioned more indirectly. Lastly, environmental exposure to SDHI residues, especially via food, could amplify clinical features in people who are already SDH-deficient.
- The existence or high probability of ecotoxic effects on non-target organisms when using SDHIs, considering that SDH is an enzyme found in many species and that there is relative conservation of the encoding sequence for SDH for certain subunits of this enzyme.
- The existence or high probability of health effects in humans or ecotoxic effects on non-target organisms, as a result of cumulative exposure to several ASs sharing the same mode of action or to multiple pollutants involving the regulation of cellular respiration.

54 Zhao T., Mu X., You Q. Succinate: An initiator in tumorigenesis and progression. Oncotarget 8 (2017) 53819-53828.

6. Hypothesis of the inadequacy of "conventional" toxicological tests for the toxicity assessment of SDHIs

According to the researchers who issued the warning, mitotoxicity is a health effect that is serious enough to be taken into consideration during the assessment of active substances, without any risk assessment and therefore based solely on the identified hazard. Such provisions exist, for example, for carcinogenicity, mutagenicity and reprotoxicity (CMR), categories 1A and 1B, and more recently for endocrine-disrupting (ED) effects. This position remains a political decision that should ultimately be adopted at EU level, and it is not for the GECU to determine the advisability of a political decision. Regarding this position, however, the GECU notes the points set out below.

Unlike with other regulations relating to the safety of chemical products, there are no regulatory definitions or guidelines in the context of Regulation (EC) No 1107/2009 enabling the "hazard equivalence" of a health effect to be estimated, in comparison with a CMR effect. This notion of equivalent level of concern can be found in the REACh Regulation[55] where it is based in particular on the examination of the following criteria:

- the possibility of serious health effects in humans,
- the irreversibility and delay of effects,
- the demonstrated impacts on quality of life and the existence of societal concern,
- the inability to define a "safe" dose.

In the absence of similar provisions for phytopharmaceutical substances, it is not clear whether there is a regulatory possibility, with guaranteed effectiveness at EU level, enabling a health effect such as a mitotoxic effect to be considered as equivalent to a CMR effect. This regulatory position for phytopharmaceutical substances results from the hypothesis that non-CMR (and non-ED) health effects can be assessed and managed based on a risk assessment, in particular by ensuring that exposure levels remain below thresholds, considered as having no health effect, appearing in assessment dossiers. In the current state of knowledge relating to SDH, there are no data confirming or contradicting the validity of this hypothesis of thresholds of enzymatic inhibition or succinate accumulation below which health effects would not occur. Nevertheless, the GECU underlines that:

- Mechanisms of toxic action involving enzymatic inhibition are considered in many regulations as "threshold" effects,
- Mechanisms of carcinogenesis relying on a mechanism of non-genotoxic action are also considered in many regulations as "threshold" effects,
- In cases of CMR or ED effects covered by special regulatory provisions, a great deal of available knowledge supports no-threshold hypotheses for several reasons including the irreversibility of mutations induced by direct genotoxic substances, the existence of vulnerability windows, and the non-linearity of dose-effect relationships.

However, Regulation (EC) No 1107/2009, under point (4) of Annex II, defines criteria for considering an approved active substance as a candidate for substitution. This status requires a comparative assessment for each use prior to any marketing authorisation decision. An active substance can be a candidate for substitution if it meets any of the following conditions:

- its acceptable daily intake (ADI), acceptable operator exposure level (AOEL) or acute reference dose (ARfD) is significantly lower than those of the majority of the approved active substances within groups of substances/use categories,

55 https://ec.europa.eu/jrc/en/publication/eur-scientific-and-technical-research-reports/identification-substances-very-high-concern-svhc-under-equivalent-level-concern-route-reach

- it meets two of the criteria to be considered as a PBT (Persistent, Bioaccumulative and Toxic) substance,
- there are reasons for concern linked to the nature of the critical effects (such as developmental neurotoxic or immunotoxic effects) which, in combination with the use/exposure patterns, amount to situations of use that could still cause concern, for example, high potential of risk to groundwater, even with very restrictive risk management measures (such as extensive personal protective equipment or very large buffer zones),
- it contains a significant proportion of non-active isomers,
- it is or is to be classified as carcinogenic or toxic to reproduction category 1A or 1B, if the substance has not been excluded,
- on the basis of the assessment of Community or internationally agreed test guidelines or other available data and information, reviewed by the Authority, it is considered to have endocrine-disrupting properties that may cause adverse effects in humans if the substance has not been excluded.

Certain SDHI active substances are included on the list of candidate substances for substitution since they meet two of the criteria (P and T) for consideration as a PBT substance. This means that a comparative assessment of these substances must be undertaken for each use during marketing authorisation reviews.

Regarding the abandonment of traditional practices involving the use of rotenone or cyanide salts for pest-control purposes, presented by the researchers issuing the warning as the consequence of their mechanism of action on cellular respiratory function, this interpretation appears hasty given that these substances have never been assessed under Regulation (EC) No 1107/2009 concerning phytopharmaceutical substances[56]. Conversely, the withdrawal or discontinuation of use of these substances within the current regulatory framework could also be viewed as reflecting this framework's effectiveness in identifying the substances posing the greatest risk. Lastly, for cyanide salts, the available data seem to suggest a much less targeted mechanism of toxic action than for SDHIs.

Regarding the possible inadequacy of the toxicological tests undertaken for the approval of active substances, the GECU notes the following points:

- The assessment of genotoxicity as part of regulatory dossiers enables the induction of gene mutations as well as structural and numerical chromosome alterations to be evaluated. This assessment requires the implementation of a battery of tests measuring these various genetic events. At the very least, these include a gene mutation test in bacteria, a gene mutation test in mammalian cells, an *in vitro* chromosomal aberration or micronucleus test and an *in vivo* chromosomal aberration or micronucleus test, usually conducted in accordance with the applicable guidelines (see Annex 2).
- Based on this standard battery of tests, it is not possible to observe or suspect carcinogenic effects not resulting from direct genotoxicity but rather, as seems to be the case for SDH-deficient patients, those resulting from an epigenetic mechanism.
- However, in order to detect carcinogenic potential even with negative genotoxicity tests, the assessment of active substances requires that carcinogenicity studies be conducted in at least two different animal species. These are usually carried out over a lifetime, in rats (two years) and mice (18 months). The European monographs for certain SDHI active substances, available online on the EFSA website, report carcinogenic effects in rats and/or mice. For authorised active substances for which cases of cancer have been reported during animal studies, the review of their approval dossiers considered either that these cancer cases were not related to a mechanism transposable to humans (for certain cases of liver and thyroid cancer) or that, in the absence of genotoxicity, they were the result of a non-genotoxic

56 Rotenone was assessed under the Biocides Regulation.

mechanism and that there is therefore a dose below which they would not occur. In this last case, critical effects occurring at lower doses were selected as the point of departure for the calculation of toxicity reference values for chronic toxicity.

- Furthermore, in addition to genotoxicity tests and carcinogenesis studies, the assessment of active substances requires at least the implementation of subchronic toxicity and reprotoxicity studies. During all of these studies, conducted in several species, multiple (biochemical, physiological, behavioural, etc.) parameters are measured, providing numerous opportunities to detect precursor effects (occurring before carcinogenesis) of significant SDH activity inhibition in treated animals, as this enzyme is present in all of the tested species. By cross-reading with other substances, it appears that these minimum regulatory tests on targeted, chronic and sub-chronic toxicity, conducted in accordance with the guidelines, are quite effective at detecting straightforward cardiac (histopathological or weight changes) or neurological effects, also encountered in SDH-deficient patients. Such effects have not been reported in the dossiers for SDHI ASs.
- Lastly, depending on the results of the minimum regulatory tests or when data are available in the scientific literature, it is possible to ask for more targeted studies, such as "mechanistic" toxicity studies, when assessing active substances. However, in the current state of the European regulations, systematically taking into account a phytopharmaceutical active substance's mechanism of toxic action in pests, except in specific cases not involving SDHIs, is not a requirement for interpreting the available data and/or requesting additional studies not included in the standard protocols.
- In the context of SDHIs, it could be worthwhile to supplement these regulatory assessments with studies characterising the affinity of SDHIs to human SDH and their inhibition kinetics, considering possible inter-species similarities or differences.

For authorised SDHIs, the available studies and conclusions have been published in the EFSA Journal[57]. Key data from these studies are summarised in the tables in Annexes 4 and 5.

Lastly, regarding the hypothesis of the inadequacy of murine models for detecting the toxicity and/or carcinogenicity of SDHIs, the GECU notes that:

- Based on the available data, the experts cannot fully agree with this conclusion since the reported data mainly involve the non-occurrence of pheochromocytoma or paraganglioma tumours in mice with heterozygous mutations in order to significantly reduce the SDH activity of their cells. It is entirely possible that other parameters, out of all those conventionally measured in mouse studies complying with the guidelines on toxicity, were disrupted (but not measured) in these studies primarily targeting carcinogenicity in genetically modified animals. It is also quite possible that the small study populations did not enable the detection of non-carcinogenic effects, taken into account in the assessment of active substances (e.g. effects on the weight of animals or certain organs, or biochemical, biological or behavioural modifications).
- Based on the available data, the possibility of rats being suitable models cannot be ruled out, and SDHIs, like all active substances, are also tested in rats.
- The embryotoxicity characteristic of animals homozygous for an SDH-deficient gene has never been observed in SDHI assessments.

57 Benzovindiflupyr: https://www.efsa.europa.eu/en/efsajournal/pub/4043
Bixafen: https://www.efsa.europa.eu/fr/efsajournal/pub/2917
Boscalid: Review report for the active substance boscalid (SANCO/3919/2007-rev. 5, 21 January 2008)
Carboxin: http://www.efsa.europa.eu/fr/efsajournal/pub/1857
Fluopyram: https://www.efsa.europa.eu/fr/efsajournal/pub/3052
Flutolanil: https://www.efsa.europa.eu/en/efsajournal/pub/rn-126
Fluxapyroxad: https://www.efsa.europa.eu/fr/efsajournal/pub/2522
Isofetamid: https://www.efsa.europa.eu/fr/efsajournal/pub/4265
Isopyrazam: https://www.efsa.europa.eu/fr/efsajournal/pub/2600
Penthiopyrad: https://www.efsa.europa.eu/fr/efsajournal/pub/3111
Sedaxane: https://www.efsa.europa.eu/fr/efsajournal/pub/2823

- The lack of murine models for disease is a potential barrier for recommending additional toxicological studies, targeting the mechanism of carcinogenic action, when assessing SDHIs (in the event that such additional studies are deemed necessary).

7. Hypothesis of health effects in humans comparable to those identified in patients carrying SDH mutations (dietary, non-dietary and occupational exposure)

Regarding food contamination, the points set out above demonstrate, for the tested SDHIs, that this contamination is far below the known regulatory limits and only represents a small fraction of the doses currently deemed to have no effect, even after taking into account the total diet. This finding is consistent with assessments of active substances during which scenarios are developed for various exposure situations (consumers, professional users, etc.), which are then compared with the appropriate toxicity reference values: if these values are exceeded, the marketing of the substances is not authorised. This finding also suggests that good agricultural practices are complied with for this class of substances.

The carcinogenic effects described in SDH-deficient patients occur in situations of long-term modifications to complex II and to induce these effects in non-mutant patients, appear to require a phenomenon of even partial irreversible SDH inhibition by exogenous compounds. This inhibition mode does not seem to be that of the SDHIs used as phytopharmaceutical active substances, which prevent the substrate from reaching the active site via steric hindrance. SDHIs all target the ubiquinone binding site located at the interface between the SDHB, -C and -D subunits[58]. Although this heterotetrameric protein structure is well conserved, protein sequences can diverge across species and lead to variable inhibition levels, especially between the fungus, plant and animal kingdoms[59]. These variations can be responsible for differences in the inhibitor's affinity for the enzyme. For example, in phytopathogenic species such as *Botrytis cinerea*, SDH is strongly inhibited by low concentrations of boscalid. In the yeast *Saccharomyces cerevisia*, this inhibition is still observed but at higher concentrations, while the mammalian enzyme from pig liver is virtually resistant[60]. These inter-species differences in sensitivity may be due to structural differences in the subunits and determine an organism's response to potential health effects.

The transposition of carcinogenesis mechanisms between patients carrying SDH mutations and patients exposed externally to inhibiting substances should also take into account exposure doses and especially internal doses in light of the results of toxicokinetic studies characterising the fate of a substance in the body. Studies undertaken within the regulatory framework of AS approval indicated that after oral administration in rats, boscalid was rapidly but only partially absorbed in the gastrointestinal tract and had an initial half-life of eight hours. Distribution was rapid in the gastrointestinal tract, liver and adipose tissue for the lowest tested dose (50 mg/kg). At a higher dose (500 mg/kg), distribution was similar in males whereas in females, boscalid was mainly distributed in the gastrointestinal tract, liver, thyroid and kidneys. These studies did not show boscalid to have cumulative potential and around 99% of the administered dose had been eliminated seven days following administration. These studies also showed this compound to be rapidly and intensively metabolised with the formation of numerous biotransformation products. The main metabolic pathway was hydroxylation of the diphenyl group. Numerous metabolites also formed via conjugation reactions. No major differences were observed between the sexes or tested doses. Similarly, the

58 Sierotzki H, Scalliet G. A review of current knowledge of resistance aspects for the next-generation succinate dehydrogenase inhibitor fungicides. Phytopathology. 103 (2013) 880-7.

59 Huang S, Millar AH. Succinate dehydrogenase: the complex roles of a simple enzyme. Curr Opin Plant Biol. 16 (2013) 344-9.

60 Monograph on nicobifen, 2002. Available online at the following address: https://www.bvl.bund.de/SharedDocs/Downloads/04_Pflanzenschutzmittel/02_eu_berichte/Boscalid-DAR.html?__blob=publicationFile&v=2

other active substances authorised in France have been the subject of metabolite characterisation and toxicokinetic studies in rodents. Key data are summarised in the table in Annex 4.

All of these data indicate rapid and extensive metabolism of the ASs and a lack of bioaccumulation in rodents. They support a relatively low internal dose in relation to external doses and encourage caution in the interpretation of data obtained *in vitro*. However, since the regulations generally do not require the provision of information regarding the enzyme-inhibiting activity of metabolites found in humans or animals, this is not documented.

The GECU also notes:

- incomplete penetrance of disease in patients carrying harmful mutations, different expression depending on the cell, a wide variety of clinical signs for apparently identical enzymatic activities, as well as *in vitro* and *in vivo* difficulty reproducing tumours associated with even high SDH deficiencies in animals[61]. All of this suggests that while lack of SDH activity and/or succinate accumulation are undeniably necessary for tumour promotion, they are not necessarily sufficient to initiate this process if they occur as isolated anomalies[44],
- that the regulation of cellular metabolism, in particular the capacity of cells to survive and develop by favouring glycolysis to the detriment of mitochondrial respiration (the Warburg effect, which seems to play a key role in the cancers described above), is a complex process involving multiple changes that represent potential targets for environmental pollutants. For example, a study demonstrated the capacity of benzo[a]pyrene, a polycyclic aromatic hydrocarbon, to induce metabolic reprogramming favouring glycolysis, described as a Warburg-like effect, *in vitro* and at the lowest tested doses[62]. More generally, cellular glycolytic activity is also modulated by activities with oxygen, substrate, iron and other enzymatic cofactor availability.

In short, the transposition of clinical observations made in patients carrying SDH mutations to people environmentally exposed to SDHIs is currently hindered by the following uncertainties for which there are few or no data:

- the sensitivity of the human enzyme to the various SDHI active substances,
- actual exposure levels in cellular targets (internal dose) in the context of extensively metabolised compounds,
- the possible effects of this isolated and *a priori* limited inhibition, in a context of strong regulation under the influence of internal and external factors.

8. Possibility of ecotoxic effects on non-target organisms when using SDHIs

The assessment of risks to environmental organisms undertaken within the regulatory framework of placing products on the market enables specific toxicity values to be established for each substance and product and helps ensure safety of use under the described conditions. Taking into account exposure in environmental organisms is a necessary condition for risk assessments with a view to MA issuance. No ecotoxic effects on non-target organisms are expected in the state of knowledge available during the approval of these active substances, subject to compliance with the conditions of use. The various acute and chronic toxicity tests set out in Regulation (EU) No 283/2013 and undertaken for active substances in the SDHI class are listed in Annex 2. These tests cover birds/mammals, aquatic organisms, soil organisms, bees and other non-target arthropods. They also cover acute, chronic and developmental exposure. In the conditions under which they have been

61 Lepoutre-Lussey C. et al. From Nf1 to Sdhb knockout: Successes and failures in the quest for animal models of pheochromocytoma. Mol Cell Endocrinol. 421 (2016) 40-8.

62 Hardonniere K. The environmental carcinogen benzo[a]pyrene induces a Warburg-like metabolic reprogramming dependent on NHE1 and associated with cell survival. Sci Rep. 6 (2016) 30776

conducted, i.e. for uses considered as representative of the active substance, these tests have led to the conclusion that SDHIs are safe to use.

As part of the *a priori* risk assessment process for non-target organisms, the establishment of toxicity values for a substance and product can enable a potential combined effect of substances (within a product) to be revealed.

None of the phytopharmacovigilance data reported above suggest that active substances in the SDHI class have been involved in effects of massive mortality in wildlife, bees or domestic animals.

Three publications dating from 2018, mentioned by the issuers of the warning and reporting SDHI effects in fish, were analysed as part of this work. The analysis is presented in Annex 6. It shows that these publications did not provide new evidence regarding the ecotoxicity of SDHIs.

Lastly, the GECU notes the lack of institutional mechanisms for the post-marketing monitoring of contamination in matrices other than water intended for human consumption, in order to validate the choice of representative uses and take into account the use of multiple treatments, especially for persistent substances such as SDHIs.

9. Hypothesis of cumulative exposure to several ASs acting on the respiratory chain, leading to health or ecotoxicological effects

The issue of cumulative exposure to several active substances and more generally to several chemical products reflects the reality of dietary, occupational and environmental exposure and is common to numerous regulated products. To date, there is no definitive response to this issue which requires additional research, especially in the field of toxicology. This lack of response is not specific to the class of SDHIs. Nevertheless, the GECU underlines that several scientific and regulatory studies are under way to take this reality into account. They are summarised below.

In addition to "substance by substance" risk assessments, the regulations dedicated to plant protection products and biocides stipulate that the possibility of cumulative effects associated with combined exposure to several substances should also be taken into account in the assessment of these products. For plant protection products and biocides containing several active substances, the cumulative risks related to their use must therefore be assessed prior to approval.

The European Chemicals Agency (ECHA), in partnership with the EU Member States, has developed a methodology for assessing risks associated with combined exposure to several biocidal substances[63]. The approach adopted relies on the concept of additivity implemented using the hazard index (HI) method. The hazard index consists in adding up the individual hazard quotients (HQs) of the various substances covered by the cumulative risk assessment. Initially, the HQ is the ratio between exposure to an individual substance and its reference value. Then, to refine the assessment, toxicity reference values are established for each target organ/system common to the various substances. The same approach has systematically been used since January 2016 for the assessment of cumulative risks associated with the use of a plant protection product containing several active substances (for acute food risks and for risks via non-dietary exposure). In the case of SDHIs for example, a combined assessment was undertaken for a mixture of fluxapyroxad and epoxiconazole.

63 ECHA: Guidance on the Biocidal Products Regulation Volume III Human Health - Assessment & Evaluation (Parts B+C), February 2017 4.4.1 “Risk Characterisation from combined exposure to several active substances or substances of concern within a biocidal product”

Major work on the development of a cumulative risk assessment methodology for pesticides in food has also been initiated at EU level under the leadership of EFSA and the European Commission and is in the process of being finalised[64]. The aim is to ultimately have a methodology based, on the one hand, on the estimation of exposure and on the other hand, on the identification of cumulative assessment groups (CAGs) for active substances that will be covered by cumulative assessments. Regarding the identification of CAGs for active substances, EFSA decided to develop an approach for assessing risks associated with exposure to multiple pesticides via food. CAGs were mainly defined, according to a phenomenological approach, for a common target organ or cell group and the same specific effect, refined when possible with a common mode/mechanism of toxicological action. At first, EFSA's Scientific Panel on Plant Protection Products and their Residues (PPR Panel) applied this methodology to define groups of toxic pesticides for the thyroid and central nervous system and therefore did not specifically consider the SDHI effects mentioned above (however, certain SDHIs were identified as enzyme inducers responsible for possible thyroid effects during animal toxicity tests).

Step-wise approaches are therefore recommended with the gradual refinement of the hazard and exposure assessment. However, the current lack of mechanistic data for establishing the modes of action underlying common specific effects limits the use of higher hazard assessment levels and the generation of such data remains a major challenge. By default, for SDHIs having a common mode of action, an initial approach based on the weighted sum of exposure could be used. In light of the food intakes documented in the most recent TDSs for the measured SDHIs, the summation approach does not seem to call into question the current conclusion.

Several European research projects are currently being undertaken to generate alternative methods for toxicological testing relying on a mechanistic approach. For example, the aim of EU-ToxRisk[65] is to develop *in vitro* tests in human cells as well as testing strategies. The objective is to drive the required paradigm shift in toxicological assessment away from a phenomenological approach using animal testing towards an approach based on human cell responses and a mechanistic understanding of chemical adverse effects. Over time, EU-ToxRisk will integrate advancements in cell biology, -omics technologies, systems biology and computational modelling to define the complex chains of events that link chemical exposure to toxic outcome.

In parallel with this work focusing on hazards, the Netherlands National Institute for Public Health and the Environment (RIVM) was commissioned by the European Commission and EFSA to develop a probabilistic model within the Monte Carlo Risk Assessment (MCRA) software, for exposure via food and drinking water. It provides for the use of CAGs and relative potency factors. It is intended to be usable for estimating cumulative (acute and chronic) exposure to pesticide residues within an *a priori* (estimated exposure for setting MRLs) or *a posteriori* (actual exposure in populations) framework. The MCRA model is also being used as part of the EuroMix (Horizon 2020) project which aims to develop an experimentally verified, tiered strategy for the risk assessment of mixtures of chemicals derived from multiple sources across different life stages. The results of the experiments undertaken will be described as practical guidance for the implementation of the future toxicological assessment strategy. Since these tools are not currently available for SDHIs or for other classes of active substances or any chemical products, the cumulative assessment of risks is not systematic.

64 EFSA 2007: Suitability of existing methodologies and, if appropriate, the identification of new approaches to assess cumulative and synergistic risks from pesticides to human health with a view to set MRLs for those pesticides in the frame of Regulation (EC) 396/2005
EFSA 2008: Risk Assessment for a Selected Group of Pesticides from the Triazole Group to Test Possible Methodologies to Assess Cumulative Effects from Exposure through Food from these Pesticides on Human Health
EFSA 2012: Guidance on the Use of Probabilistic Methodology for Modelling Dietary Exposure to Pesticide Residues
EFSA 2013: Identification of pesticides to be included in cumulative assessment groups on the basis of their toxicological profile
EFSA 2013: Relevance of dissimilar mode of action and its appropriate application for cumulative risk assessment of pesticides residues in food
EFSA 2018: Public consultation on the establishment of cumulative assessment groups of pesticides for their effects on the nervous system
EFSA 2018: Public consultation on MIXTOX Guidance

65 http://www.eu-toxrisk.eu/page/en/about-eu-toxrisk.php

Lastly, in 2017, ANSES published a scientific and technical support note on the feasibility of establishing an overall maximum limit for pesticides in food with a view to protecting consumers from the cumulative effect of these substances. This opinion revealed that:

- The establishment of an "overall" MRL would reduce the assessment of exposure to a substance or group of substances to the sole measurement of exposure levels, without fully integrating the notion of related risk, which alone ensures the protection of human health. This notion of a single limit in foods could only be relevant if the target was the absence of all residues in foodstuffs,
- The main challenges related to the establishment of an overall MRL for food therefore lie firstly in the very identification of the level to be set for this limit and secondly in the justification of its integration in the harmonised European and international system on the basis of health reasons,
- The development of cumulative risk assessment methodologies, currently in progress at EU level, should be accelerated by further mobilising the scientific community. This work should lead to the introduction of common, harmonised methodologies for assessing cumulative risks at the EU and international levels.

The assessment of risks to environmental organisms within the regulatory framework of placing products on the market is carried out based on toxicity data specific to each active substance and each product. Thus, in the event of a product containing several active substances, a potential combined effect of the substances may be observed. Furthermore, beyond the establishment of toxicity values specific to each group of environmental organisms, the required studies also include observations about the behaviour of individuals. Any abnormal behaviour (e.g. in the swimming performance of fish, or avoidance behaviour in earthworms) is recorded and taken into account in risk assessments.

EFSA has also initiated work to propose harmonised methodologies for assessing risks related to combined exposure to multiple chemicals for all relevant areas within EFSA's remit, i.e. human health, animal health and ecological areas[66].

10. Conclusions of the Working Group

10.1 Opinion of the GECU on the scientific hypotheses identified by the issuers of the warning

In light of the information set out above, the GECU notes that:

- It is not possible to draw definitive conclusions regarding all of the issues and hypotheses identified by the researchers issuing the warning,
- Some of these issues and hypotheses involve points common to all phytopharmaceutical active substances and other regulated chemicals: regulatory context not providing for the exclusion of substances on the basis of hazards with the exception of CMR/ED substances, risk management via the application of toxicity thresholds, cumulative exposure, and the predictive nature of ecotoxicology tests for persistent substances. Due to their applicability to all phytopharmaceutical substances, these issues do not constitute an alert specific to the class of SDHIs,

66 EFSA Scientific Committee, Hardy A, et al. Draft guidance on harmonised methodologies for human health, animal health and ecological risk assessment of combined exposure to multiple chemicals. Document available for public consultation at the following address: https://www.efsa.europa.eu/sites/default/files/consultation/consultation/180626-1-ax1.pdf

- On the other hand, some other issues and hypotheses are more specific to the class of SDHIs: cross-sensitivity of human and fungal enzymes, introduction of targeted studies in addition to the minimal regulatory studies to consider mitotoxic risks, and the relevance of carcinogenesis studies in rodents for detecting cancers attributable to impaired SDH function.

However, these remaining uncertainties should be read in light of the following information:

- Apparent compliance with good agricultural practices for this class of substances, demonstrated by numerous analyses finding the maximum residue limits (MRLs) to be exceeded only in a few exceptional cases and probably sustained by the need to limit the emergence of fungal resistance (see Annex 7),
- Low levels of total dietary exposure in relation to the current toxicological thresholds based on a wide range of tests including carcinogenicity tests in rats,
- Rapid metabolism of these substances leading to low internal doses in relation to external exposure,
- The current state of scientific knowledge regarding the plausibility of a carcinogenic effect of SDH inhibition likely to be reversible and/or limited succinate accumulation,
- The absence, in the current state of the data brought to its attention, of any real signs of a health alert in terms of specific effects observed for environmental organisms,
- The absence, in the current state of the data brought to its attention, of any real signs of a health alert in terms of an increase in the incidence of specific cancers associated with SDH-deficiency in humans not carrying a mutation (in exposed workers, for example), despite the fact that some of these compounds have been on the market for a long time.

The GECU therefore considers, based on data from the literature, European assessments of the substances and vigilance data, that the scientific information and hypotheses mentioned by the issuers of the warning:

- do not provide any evidence of exposure not taken into account in the assessments of the active substances in question,
- highlight residual uncertainties relating to risks that may not have been taken into account in the assessments of the active substances in question. In the absence of any signs of a health alert, these uncertainties justify the recommendations made in the following paragraph.

10.2 Recommendations of the GECU

In order to resolve certain remaining uncertainties highlighted during the examination of the scientific hypotheses identified by the issuers of the warning, and more broadly to make phytopharmaceutical active substances safer to use, the GECU is issuing the following recommendations, which have been grouped together by theme. These recommendations should be shared at European level, in accordance with the procedures for assessing active substances. Some of them call for the provision of new knowledge, possibly requiring that the safety of use of SDHI active substances be reassessed as knowledge is produced.

To better characterise the hazards associated with SDHI active substances:

- Characterise the inhibition properties of SDHIs and their metabolites and by-products on human enzymes, using appropriate tests and considering combinations of active substances with the same mechanism of action. These inhibition properties should be compared with estimated internal exposure levels for consumers,

- Characterise the inhibition properties of SDHIs and their metabolites and by-products on enzymes of non-target organisms. These inhibition properties should be compared with estimated exposure levels for these organisms,

- Develop the use of detection and characterisation tools for mitotoxic effects that can be used in regulatory assessments.

To better characterise exposure:

- Continue to implement surveillance and control plans providing objective information about actual exposure in the population and in environmental organisms and enabling the data contained in authorisation dossiers to be highlighted,
- Include other SDHI active substances in surveillance and control plans and in future French Total Diet Study work, then update the resulting *a posteriori* risk assessments,
- Take into account airborne exposure when such data are available, in particular for boscalid which was the only SDHI selected in the expert appraisal on the definition of methods of monitoring pesticides in air.

To better characterise the risks associated with active substances, including SDHIs:

- Test the feasibility of retrospectively and prospectively monitoring changes in the incidence of known diseases involving "SDH" mutations (registries),
- Quantify internal exposure for exposed workers and consumers,
- Carry out work to improve the sensitivity of toxicological and ecotoxicological tests relating to the mechanisms of action of active substances,
- Carry out expert appraisal and research work on cumulative exposure for a given effect, also taking into account common mechanisms of toxic action. In the specific case of SDHIs, this approach should also be applied to combinations of fungicides inhibiting mitochondrial respiration, in particular to document the expected effect in human cells,
- Continue efforts aimed at creating, collecting and interpreting phytopharmacovigilance data in order to detect potential warning signals involving the use of products throughout France,
- Promote the development and use of adverse outcome pathways (AOPs)[67] to consider the combined effects of mixtures[68].

To reinforce the current regulatory schemes:

- Introduce regulatory requirements on the relevance to humans and non-target organisms of the active substances' mechanisms of pesticide action, provided that the target is known and present in humans and/or non-target organisms,
- Consider the possibility of identifying, as with genotoxicity, toxic effects potentially justifying a precautionary approach similar to that described in the regulations applicable to CMR substances,
- Consider using complex ecotoxicological tests simulating natural conditions (cosms) on a more systematic basis,
- Consider the possibility of regularly monitoring non-aqueous matrices (soil in particular) in order to document concentrations of persistent active substances and metabolites and assess the possible cumulative ecotoxic risk after they have been placed on the market,
- Continue the integration of cumulative approaches in regulatory assessment processes.

Date of validation of the collective expert appraisal report by the Working Group: 13/12/2018

67 Sequences of events leading to the occurrence of an *in vivo* adverse effect, based on the chemical structure of a target chemical or a group of similar chemicals and the molecular initiating event

68 Souders CL 2nd, Liang X, Wang X, Ector N, Zhao YH, Martyniuk CJ. High-throughput assessment of oxidative respiration in fish embryos: Advancing adverse outcome pathways for mitochondrial dysfunction. Aquat Toxicol. 199 (2018) 162-173.

ANNEXES

Annex 1: Internal request decision

This Annex is a translation of the original French version. In the event of any discrepancy or ambiguity the French language text shall prevail.

Decision No. 2018-05-144

INTERNAL REQUEST

The Director General of the French Agency for Food, Environmental and Occupational Health & Safety (ANSES),

Having regard to the French Public Health Code, and in particular its Article L. 1313-3 giving ANSES the prerogative to issue an internal request on any question with a view to accomplishing its missions,

Has decided the following:

Article 1. The French Agency for Food, Environmental and Occupational Health & Safety is issuing an internal request to conduct an expert appraisal whose characteristics are listed below.

1.1 Themes and objectives of the expert appraisal

The objective is to determine, based on data from the literature, European assessments of the substances and phytopharmacovigilance data, whether the scientific information and hypotheses mentioned by the authors of an article on the potential health risks of using succinate dehydrogenase inhibitor (SDHI) fungicides in agriculture have provided any evidence of exposure or risks that were not taken into account in the assessments of the fungicidal active substances in question.

1.2 Background of the internal request

In an article published on 16 April 2018 in the press, several scientists drew attention to the potential health risks of using succinate dehydrogenase inhibitor (SDHI) fungicides in agriculture. In this context, ANSES is calling on its experts to consider all the available scientific data on this subject and, in particular, to immediately examine the information mentioned by the scientists issuing the warning. The analysis of this warning signal will be entrusted to a group of experts.

1.3 Questions on which the expert appraisal work will focus

- Based on data from the literature and phytopharmacovigilance data, do the scientific information and hypotheses mentioned by the issuers of the warning provide any evidence of exposure or risks not taken into account in the assessments of the active substances in question?
- If new evidence is found, should it be presented at European level and, if appropriate, should immediate risk management measures be taken for authorised products containing these substances?
- Issue recommendations for follow-up action in response to this warning.

1.4 Estimated duration of the expert appraisal

Three months

Article 2. An opinion will be issued and published by the Agency following completion of the work.

Signed in Maisons-Alfort on 24 May 2018

Dr Roger Genet
Director General

Annex 2: List of relevant test methods and guidelines for implementation of Regulation (EU) No 283/2013

- Toxicological and metabolism studies

Reference to Part A of the Annex to Regulation (EU) No 283/2013	Test method	Comments
5.1. Studies on absorption, distribution, metabolism and excretion in mammals		
5.1.1. Absorption, distribution, metabolism and excretion after exposure by oral route	OECD Test No. 417: Toxicokinetics	Generally undertaken in rats, unless another species appears more sensitive and more relevant to humans
Comparative *in vitro* metabolism studies on animal species to be used in pivotal studies and on human material (microsomes or intact cell systems)	No validated guidelines Protocols available on the ECVAM website: https://ecvam-dbalm.jrc.ec.europa.eu/	
5.1.2. Absorption, distribution, metabolism and excretion after exposure by other routes	OECD Test No. 417: Toxicokinetics	E.g. study by inhalation if the active substance is in gaseous form
Acute toxicity		
5.2.1. Oral	OECD Test No. 420: Acute Oral Toxicity - Fixed Dose Procedure OECD Test No. 423: Acute Oral Toxicity - Acute Toxic Class Method OECD Test No. 425: Acute Oral Toxicity - Up-and-Down Procedure OECD Test No. 401: Acute Oral Toxicity (if undertaken before December 2002)	
5.2.2. Dermal	OECD Test No. 402: Acute Dermal Toxicity	

Reference to Part A of the Annex to Regulation (EU) No 283/2013	Test method	Comments
5.2.3. Inhalation	OECD Test No. 403: Acute Inhalation Toxicity OECD Test No. 436: Acute Inhalation Toxicity - Acute Toxic Class Method	
5.2.4. Skin irritation	OECD Test No. 404: Acute Dermal Irritation/Corrosion OECD Test No. 431: In Vitro Skin Corrosion: Human Skin Model Test OECD Test No. 430: In Vitro Skin Corrosion: Transcutaneous Electrical Resistance Test Method (TER) OECD Test No. 435: In Vitro Membrane Barrier Test Method for Skin Corrosion OECD Test No. 439: In Vitro Skin Irritation: Reconstructed Human Epidermis Test Method	Sequential approach to limit testing in vertebrates
5.2.5. Eye irritation	OECD Test No. 405: Acute Eye Irritation/Corrosion OECD Test No. 437: Bovine Corneal Opacity and Permeability Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage OECD Test No. 438: Isolated Chicken Eye Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage	Sequential approach to limit testing in vertebrates
5.2.6. Skin sensitisation	OECD Test No. 429: Skin Sensitisation OECD Test No. 406: Skin Sensitisation - Local Lymph Node Assay OECD Test No. 442A: Skin Sensitisation - Local Lymph Node Assay: DA OECD Test No. 442B: Skin Sensitisation - Local Lymph Node Assay: BrdU-ELISA	Local lymph node assay to be favoured
5.2.7. Phototoxicity	OECD Test No. 432: In Vitro 3T3 NRU Phototoxicity Test	

Reference to Part A of the Annex to Regulation (EU) No 283/2013	Test method	Comments
5.3. Short-term toxicity		
5.3.1. Oral 28-day study	OECD Test No. 407: Repeated Dose 28-day Oral Toxicity Study in Rodents	
5.3.2. Oral 90-day study	OECD Test No. 408: Repeated Dose 90-day Oral Toxicity Study in Rodents OECD Test No. 409: Repeated Dose 90-day Oral Toxicity Study in Non-Rodents	Tested species: rats and dogs (and possibly mice)
5.3.3. Other routes	OECD Test No. 410: Repeated Dose Dermal Toxicity: 21/28-day Study OECD Test No. 411: Subchronic Dermal Toxicity: 90-day Study OECD Test No. 412: Subacute Inhalation Toxicity: 28-day Study OECD Test No. 413: Subchronic Inhalation Toxicity: 90-day Study	Tests by inhalation undertaken if the active substance is a gas
5.4. Genotoxicity testing		
5.4.1. In vitro studies	OECD Test No. 471: Bacterial Reverse Mutation Test OECD Test No. 476: In Vitro Mammalian Cell Gene Mutation Tests using the Hprt and xprt genes OECD Test No. 490: In Vitro Mammalian Cell Gene Mutation Tests Using the Thymidine Kinase Gene OECD Test No. 473: In Vitro Mammalian Chromosomal Aberration Test OECD Test No. 487: In Vitro Mammalian Cell Micronucleus Test Comet assay can be used if justified (no validated guidelines)	Sequential approach: Three *in vitro* tests: • One gene mutation test in bacteria • One gene mutation test in mammalian cells • One micronucleus or chromosomal aberration test in mammalian cells At least an *in vivo* micronucleus test if all *in vitro* tests are negative Otherwise, tests required for the *in vivo* exploration of the *in vitro* warning signal

Reference to Part A of the Annex to Regulation (EU) No 283/2013	Test method	Comments
5.4.2. In vivo studies in somatic cells	OECD Test No. 474: Mammalian Erythrocyte Micronucleus Test OECD Test No. 475: Mammalian Bone Marrow Chromosomal Aberration Test OECD Test No. 486: Unscheduled DNA Synthesis (UDS) Test with Mammalian Liver Cells in vivo OECD Test No. 488: Transgenic Rodent Somatic and Germ Cell Gene Mutation Assays OECD Test No. 489: In Vivo Mammalian Alkaline Comet Assay	
5.4.3. In vivo studies in germ cells	OECD Test No. 483: Mammalian Spermatogonial Chromosomal Aberration Test OECD Test No. 488: Transgenic Rodent Somatic and Germ Cell Gene Mutation Assays	
5.5. Long-term toxicity and carcinogenicity		
5.5. Long-term toxicity and carcinogenicity	OECD Test No. 451: Carcinogenicity Studies OECD Test No. 452: Chronic Toxicity Studies OECD Test No. 453: Combined Chronic Toxicity/Carcinogenicity Studies	Tested species: rat and mouse
5.6. Reprotoxicity		
5.6.1. Generational studies	OECD Test No. 416: Two-Generation Reproduction Toxicity OECD Test No. 443: Extended One-Generation Reproductive Toxicity Study	Tested species: rat
5.6.2. Developmental toxicity studies	OECD Test No. 414: Prenatal Developmental Toxicity Study OECD Test No. 426: Developmental Neurotoxicity Study	Tested species: rat and rabbit

Reference to Part A of the Annex to Regulation (EU) No 283/2013	Test method	Comments
5.7. Neurotoxicity studies		
5.7.1. Neurotoxicity studies in rodents	OECD Test No. 424: Neurotoxicity Study in Rodents	Single- or repeated-dose studies, alone or combined with a general toxicity study
5.7.2. Delayed polyneuropathy studies	OECD Test No. 418: Delayed Neurotoxicity of Organophosphorus Substances Following Acute Exposure OECD Test No. 419: Delayed Neurotoxicity of Organophosphorus Substances: 28-day Repeated Dose Study	Targeted studies for cholinesterase inhibitors
5.8. Other toxicological studies		
5.8.1. Toxicity studies of metabolites		Existence of a guidance document for the assessment of metabolites found in groundwater EU Guidance Document on the assessment of the relevance of metabolites in groundwater of substances regulated under Council Directive 91/414/EEC (SANCO/221/2000 – rev.10. final)
5.8.2. Supplementary studies on the active substance		Mechanistic studies exploring a mode of action and its relevance to humans
5.8.3. Endocrine disrupting properties	OECD Test No. 493: Performance-Based Test Guideline for Human Recombinant Estrogen Receptor (hrER) In Vitro Assays to Detect Chemicals with ER Binding Affinity OECD Test No. 455: Performance-Based Test Guideline for Stably Transfected Transactivation In Vitro Assays to Detect Estrogen Receptor Agonists and Antagonists OECD Test No. 456: H295R Steroidogenesis Assay OECD Test No. 457: BG1Luc Estrogen Receptor Transactivation Test Method for Identifying Estrogen Receptor Agonists and Antagonists	Existence of an EFSA/ECHA guidance document for the identification of endocrine disruptors Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009 Pre-publication version drafted by EFSA and ECHA staff, with support from JRC 07 June 2018

Reference to Part A of the Annex to Regulation (EU) No 283/2013	Test method	Comments
	OECD Test No. 458: Stably Transfected Human Androgen Receptor Transcriptional Activation Assay for Detection of Androgenic Agonist and Antagonist Activity of Chemicals OECD Test No. 440: Uterotrophic Bioassay in Rodents OECD Test No. 441: Hershberger Bioassay in Rats OCSPP Guideline 890.1500: Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Male Rats Assay OCSPP Guideline 890.1450: Pubertal Development and Thyroid Function in Intact Juvenile/Peripubertal Female Rats Assay US Environmental Protection Agency (2007): 15-Day Intact Adult Male Rat Assay	
5.9. Medical data		Medical surveillance of manufacturing plant personnel and monitoring studies Clinical cases Epidemiological studies
Systematic review of the published literature		Available guidance document GUIDANCE OF EFSA Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009 EFSA Journal 2011;9(2):2092

- Ecotoxicological studies

Reference to Part A of the Annex to Regulation (EU) No 283/2013	Test method	Comments
8.1.1. Effects on birds		
8.1.1.1. Acute oral toxicity to birds	OECD Test Guideline No. 223: Avian Acute Oral Toxicity Test	
	OECD Test Guideline No. 223: Avian Acute Oral Toxicity Test (updated version of July 2016)	
	US EPA OCSPP 850.2100: Avian Acute Oral Toxicity Test	
8.1.1.2. Short-term dietary toxicity to birds	OECD Test Guideline No. 205: Avian Dietary Toxicity Test	
	US EPA OCSPP 850.2200: Avian Dietary Toxicity Test	
8.1.1.3. Sub-chronic and reproductive toxicity to birds	OECD Test Guideline No. 206: Avian Reproduction Test	
	US EPA OCSPP 850.2300: Avian Reproduction Test	
8.1.2.1. Acute oral toxicity to mammals	see 5.2.1	
8.1.2.2. Long-term and reproductive toxicity to mammals	see 5.5 and 5.6	
8.1.3 Active substance bioconcentration in prey of birds and mammals (see 8.2.2.3.)		
8.1.4. Effects on terrestrial vertebrate wildlife (birds, mammals, reptiles and amphibians)	OECD Test Guideline No. 231: Amphibian Metamorphosis Assay	
8.2. Effects on aquatic organisms		
8.2.1. Acute toxicity to fish	OECD Test Guideline No. 203: Fish, Acute Toxicity Test	
8.2.2. Long-term and chronic toxicity to fish		
8.2.2.1. Fish early life stage toxicity test	OECD Test Guideline No. 210: Fish, Early-Life Stage Toxicity Test	

Reference to Part A of the Annex to Regulation (EU) No 283/2013	Test method	Comments
8.2.2.2. Fish full life cycle test	US EPA protocol OCSPP 850.1500 Fish Life Cycle Toxicity	
8.2.2.3. Bioconcentration in fish	OECD Test Guideline No. 305: Bioaccumulation in Fish: Aqueous and Dietary Exposure (as updated in October 2012)	
8.2.3. Endocrine disrupting properties	OECD Test Guideline No. 319A: Determination of in vitro intrinsic clearance using cryopreserved rainbow trout hepatocytes (RT-HEP)	
	OECD Test Guideline No. 319B: Determination of in vitro intrinsic clearance using rainbow trout liver S9 sub-cellular fraction (RT-S9)	
	OECD Test Guideline No. 229: Fish Short Term Reproduction Assay	
	OECD Test Guideline No. 230: 21-day Fish Assay: A Short-Term Screening for Oestrogenic and Androgenic Activity, and Aromatase Inhibition	
	OECD Test Guideline No. 231: Amphibian Metamorphosis Assay	
	OECD Test Guideline No. 234: Fish Sexual Development Test	
	OECD Test Guideline No. 240: Medaka Extended One-Generation Reproduction Test	
	Method C.52 Medaka Extended One Generation Reproduction Test (MEOGRT) (Annex of Regulation (EC) No 440/2008, as amended by the 8th ATP)	
	OECD Test Guideline No. 241: Larval Amphibian Growth and Development Assay	
	Method C.53 The Larval Amphibian Growth and Development Assay (LAGDA) (Annex of Regulation (EC) No 440/2008, as amended by the 8th ATP)	

Reference to Part A of the Annex to Regulation (EU) No 283/2013	**Test method**	**Comments**
8.2.4. Acute toxicity to aquatic invertebrates		
8.2.4.1. Acute toxicity to *Daphnia magna*	OECD Test Guideline No. 202: *Daphnia sp.* Acute Immobilisation Test	
8.2.4.2. Acute toxicity to an additional aquatic invertebrate species	US EPA OCSPP 850.1035 Mysid Acute Toxicity Test	
	OECD Test Guideline No. 235: *Chironomus sp*., Acute Immobilisation Test	
8.2.5. Long-term and chronic toxicity to aquatic invertebrates		
8.2.5.1. Reproductive and development toxicity to *Daphnia magna*	OECD Test Guideline No. 211: *Daphnia magna* Reproduction Test	
8.2.5.2. Reproductive and development toxicity to an additional aquatic invertebrate species	US EPA OCSPP 850.1350 Mysid Chronic Toxicity Test	
8.2.5.3. Development and emergence in *Chironomus riparius*	OECD Test Guideline No. 219: Sediment-Water Chironomid Toxicity Using Spiked Water	
	OECD Test Guideline No. 218: Sediment-Water Chironomid Toxicity Using Spiked Sediment	
	OECD Test Guideline No. 233: Sediment-Water Chironomid Life-Cycle Toxicity Test Using Spiked Water or Spiked Sediment	
8.2.5.4. Sediment dwelling organisms	OECD Test Guideline No. 218: Sediment-Water Chironomid Toxicity Using Spiked Sediment	
8.2.6. Effects on algal growth		
8.2.6.1. Effects on growth of green algae	OECD Test Guideline No. 201: Alga, Growth Inhibition Test	
8.2.6.2. Effects on growth of an additional algal species	OECD Test Guideline No. 221: *Lemna sp.* Growth Inhibition Test	

Reference to Part A of the Annex to Regulation (EU) No 283/2013	**Test method**	**Comments**
8.2.7. Effects on aquatic macrophytes	ASTM E1913-04: Standard Guide for Conducting Static, Axenic, 14-Day Phytotoxicity Tests in Test Tubes with the Submersed Aquatic Macrophyte, *Myriophyllum sibiricum Komarov*	
	OECD Test Guideline No. 238: Sediment-Free *Myriophyllum Spicatum* Toxicity Test	
8.2.8. Further testing on aquatic organisms	OECD Test Guideline No. 239: Water-Sediment *Myriophyllum Spicatum* Toxicity Test	
8.3. Effect on arthropods		
8.3.1. Effects on bees	EPPO Standard PP1/170 (4): Test methods for evaluating the side-effects of plant protection products on honeybees	
8.3.1.1. Acute toxicity to bees		
8.3.1.1.1. Acute oral toxicity	OECD Test Guideline No. 213: Honeybees, Acute Oral Toxicity Test	
	EPPO Standard PP1/170 (4): Test methods for evaluating the side-effects of plant protection products on honeybees	
8.3.1.1.2. Acute contact toxicity	OECD Test Guideline No. 214: Honeybees, Acute Contact Toxicity Test	
	EPPO Standard PP1/170 (4): Test methods for evaluating the side-effects of plant protection products on honeybees	
8.3.1.2. Chronic toxicity to bees	OECD Test Guideline No. 245: Honey Bee (*Apis mellifera* L.), Chronic Oral Toxicity Test (10-Day Feeding)	
8.3.1.3. Effects on honeybee development and other honeybee life stages	OECD Test Guideline No. 237: Honey Bee (*Apis mellifera*) Larval Toxicity Test, Single Exposure	
	OECD Series on Testing & Assessment No. 239: Guidance Document on Honey Bee Larval Toxicity Test following Repeated Exposure	

Reference to Part A of the Annex to Regulation (EU) No 283/2013	Test method	Comments
8.3.1.4. Sub-lethal effect	Oomen PA, de Ruijter A and van der Steen J, 1992. Method for honeybee brood feeding tests with insect growth-regulating insecticides. Bulletin OEPP/EPPO Bulletin 22, 613-616.	
	OECD Series on Testing & Assessment No. 75: Guidance Document on the Honey Bee (*Apis mellifera* L.) Brood Test Under Semi-Field Conditions	
	EPPO Standard PP1/170 (4): Test methods for evaluating the side-effects of plant protection products on honeybees	
8.3.2. Effects on non-target arthropods other than bees	M.P. Candolfi, S. Blümel, R. Forster et al. (2000): Guidelines to evaluate side-effects of plant protection products to non-target arthropods. IOBC, BART and EPPO Joint Initiative. ISBN: 92-9067-129-7.	
8.3.2.1. Effects on *Aphidius rhopalosiphi* 8.3.2.2. Effects on *Typhlodromus pyri*	M.P. Candolfi, S. Blümel, R. Forster et al. (2000): Guidelines to evaluate side-effects of plant protection products to non-target arthropods. IOBC, BART and EPPO Joint Initiative. ISBN: 92-9067-129-7	
8.4. Effects on non-target soil meso- and macrofauna		
8.4.1. Earthworm – sub-lethal effects	OECD Test Guideline No. 222: Earthworm Reproduction Test (*Eisenia fetida/Eisenia andrei*) (updated version of July 2016)	
8.4.2. Effects on non-target soil meso- and macrofauna (other than earthworms)	OECD Test Guideline No. 232: Collembolan Reproduction Test in Soil (updated version of July 2016)	
8.4.2.1. Species level testing	OECD Test Guideline No. 226: Predatory mite (*Hypoaspis (Geolaelaps) aculeifer*) reproduction test in soil	
8.5. Effects on soil nitrogen transformation	OECD Test Guideline No. 216: Soil Microorganisms: Nitrogen Transformation Test	
8.6. Effects on terrestrial non-target higher plants	OECD Test Guideline No. 208: Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test	

Reference to Part A of the Annex to Regulation (EU) No 283/2013	Test method	Comments
8.6.2. Testing on non-target plants	OECD Test Guideline No. 227: Terrestrial Plant Test: Vegetative Vigour Test	
8.8. Effects on biological methods for sewage treatment	OECD Test Guideline No. 209: Activated Sludge, Respiration Inhibition Test	
Systematic review of the published literature	Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009 EFSA Journal 2011;9(2):2092	

Annex 3: List of uses of authorised products in France containing a succinate dehydrogenase inhibitor (SDHI) active substance

Use no.[69]	Title
12103201	Almond trees * Foliar application[70] * Leaf curl
12103202	Almond trees * Foliar application* *Coryneum* and *Polystigma*
12103203	Almond trees * Foliar application* Brown rots (*Monilia* sp.)
14053200	Trees and shrubs * Foliar application* Miscellaneous diseases (1)
14053204	Trees and shrubs* Foliar application * Powdery mildew
16153203	Asparagus * Foliar application * *Stemphylium* sp.
16153201	Asparagus * Foliar application * Rust(s)
00106014	Oat * Foliar application * Ear disease (*Microdochium* sp.)
00106013	Oat * Foliar application * Ear disease (*Fusarium* sp.)
15103206	Oat * Foliar application * Powdery mildew
15103230	Oat * Foliar application * Eyespot
15103231	Oat * Foliar application * Crown rust
00106011	Oat * Foliar application * Septoria leaf spot
15101255	Oat * Seed Treatment * Fungi not in the Pythiaceae family
13153201	Banana trees* Foliar application * Black sigatoka
00108036	Wheat * Foliar application * Ear disease (*Microdochium* sp.)
15103202	Wheat * Foliar application * Ear disease (*Fusarium* sp.)
00108034	Wheat * Foliar application * Leaf and glume blotch tan spot
15103209	Wheat * Foliar application * Powdery mildew
15103210	Wheat * Foliar application * Eyespot
15103211	Wheat * Foliar application * Sharp eye-spot, take-all (*Rhizoctonia* sp.)
15103214	Wheat * Foliar application * Rust(s)
15103221	Wheat * Foliar application * *Septoria* leaf spot
15101201	Wheat * Seed treatment * Fungi not in the Pythiaceae family

69 The "use number" is codified in the official French Catalogue of uses of plant protection products: https://info.agriculture.gouv.fr/gedei/site/bo-agri/instruction-2015-253 and https://info.agriculture.gouv.fr/gedei/site/bo-agri/instruction-2015-253/telechargement. A "use" usually comprises three elements: *crop or plant * type of application * target pathogen*. The Latin name(s) of the target pathogen(s) is (are) usually indicated in the Catalogue. Otherwise, reference should be made to the European and Mediterranean Plant Protection Organisation's Global Database, https://gd.eppo.int/.

70 According to the context and type or size of plant, "foliar application" may encompass treatment of *all* the above-ground parts, that is, leaves, shoots, twigs, stems, branches and trunk.

Use no.[69]	**Title**
16203203	Carrot * Foliar application * Brown leaf spot
16203201	Carrot * Foliar application * Powdery mildew
16203207	Carrot * Foliar application * Grey mould and *Sclerotinia*
12153204	Blackcurrant * Foliar application * Foliage diseases
12153202	Blackcurrant * Foliar application * Powdery mildew
12153208	Blackcurrant * Foliar application * Grey mould
15101901	Straw-based cereals * Seed treatment * Crow repellent
12203208	Cherry * Foliar application * Brown rots (*Monilia* sp.)
16361202	Witloof chicory[71] * Leaf production Seedling seed treatment * Fungi not in the Pythiaceae family
00516023	Flowering brassica * Foliar application * Bacterial diseases
00516026	Flowering brassica * Foliar application * Brown leaf spot
00517025	Head brassica * Foliar application * Brown leaf spot
16323203	Cucumber * Foliar application * Powdery mildew
16342203	Cucumber * Soil treatment * Fungi not in the Pythiaceae family
16322501	Cucumber * Soil treatment * Nematodes
15203204	Oilseed brassicas * Foliar application * *Cylindrosporium* leaf spot
15203201	Oilseed brassicas * Foliar application * Fungal diseases of the siliques family
15203207	Oilseed brassicas * Foliar application * Powdery mildew
15203203	Oilseed brassicas * Foliar application * *Phoma*
15203202	Oilseed brassicas * Foliar application * *Sclerotinia*
17403202	Floral crops and green plants * Foliar application * Powdery mildew
17403201	Floral crops and green plants * Foliar application * Grey mould
16553207	Strawberry * Foliar application * Brown leaf spot
16553205	Strawberry * Foliar application * Powdery mildew
16553201	Strawberry * Foliar application * Grey mould and *Sclerotinia*
12353206	Raspberry * Foliar application * Foliage diseases
12353204	Raspberry * Foliar application * Powdery mildew
12353205	Raspberry * Foliar application * Grey mould
16851206	Legume crops Seed treatment Fungi not in the Pythiaceae family

71 *Cichorium intybus* var. *foliosum*

Use no.[69]	**Title**
15301201	Fodder grasses * Seed treatment * Fungi not in the Pythiaceae family
00518010	Fresh beans without pods Foliar application * Grey mould and *Sclerotinia*
00516015	Fresh beans and peas with pods Foliar application * Grey mould and *Sclerotinia*
16563202	Beans Foliar application* Grey mould and *Sclerotinia* (1)
16703208	Lettuce Foliar application * Brown leaf spot diseases
16603201	Lettuce * Foliar application * Grey mould and *Sclerotinia*
15451202	Fodder legumes * Seed treatment * Fungi not in the Pythiaceae family
15503201	Flax * Foliar application * *Phoma*
16661202	Sweetcorn * Seedling seed treatment * Fungi not in the Pythiaceae family
16661901	Sweetcorn * Seed treatment * Crow repellent
00120037	Maize * Seed treatment * Fungi not in the Pythiaceae family
15551202	Maize * Seed treatment * Maize head smut (1)
15551901	Maize * Seed treatment * Crow repellent
16753205	Melon * Foliar application * Powdery mildew
16752205	Melon Soil treatment * Fungi not in the Pythiaceae family
16752501	Melon * Soil treatment * Nematodes
00211002	Hazelnut trees * Foliar application * Anthracnosis
12453202	Walnut trees * Foliar application * *Ophiognomonia leptostyla*
16803204	Onion * Foliar application * Grey mould and *Sclerotinia*
16053201	Onion * Foliar application * Rust(s)
00121016	Barley * Foliar application * Ear disease (*Microdochium* sp.)
00121015	Barley * Foliar application * Ear disease (*Fusarium* sp.)
15103226	Barley * Foliar application * Net blotch and *Ramularia* leaf spot
15103225	Barley * Foliar application * Powdery mildew
15103207	Barley * Foliar application * Eyespot
15103229	Barley * Foliar application * Leaf blotch/scald
15103205	Barley * Foliar application * Rust(s)
15101245	Barley * Seed treatment * Fungi not in the Pythiaceae family
12553233	Peach trees * Foliar application * Brown rot(s) (*Monilia* sp.)
12553224	Peach trees * Foliar application * Powdery mildew
12553208	Peach trees * Foliar application * Rust(s)

Use no.[69]	**Title**
16843203	Leek * Foliar application Purple blotch
16843201	Leek * Foliar application * *Phytophthora porri*
00517100	Fresh peas without pods * Foliar application * Grey mould and *Sclerotinia*
16851201	Peas * Seedling seed treatment * Fungi not in the Pythiaceae family (1)
16863203	Sweet pepper * Foliar application * Powdery mildew
16863201	Sweet pepper * Foliar application * Grey mould and *Sclerotinia*
16862202	Sweet pepper * Soil treatment * Fungi not in the Pythiaceae family
16862501	Sweet pepper * Soil treatment * Nematodes
01141024	Potato * Soil treatment * Fungi not in the Pythiaceae family
15651203	Potato * tuber treatment * Fungi not in the Pythiaceae family
12603212	Apple trees * Foliar application * Storage diseases
12603202	Apple trees * Foliar application * Powdery mildew
12613208	Apple trees * Foliar application * *Stemphylium* brown spot
12603203	Apple trees * Foliar application * Scab
00607005	Seed crops - Sugar and fodder beet * Foliar application * Leaf spot diseases
10993207	Seed crops - Forage and lawn grasses * Foliar application * Leaf spot diseases
10993208	Seed crops - Forage and lawn grasses * Foliar application * Rust(s)
00604006	Seed crops - Fodder legumes * Foliar application * *Sclerotinia* and *Botrytis* diseases
00606004	Seed crops - Herbs, spices and medicinal crops * Foliar application * *Sclerotium*, *Sclerotinia* and *Botrytis* diseases
10993214	Seed crops - Herbs, spices and medicinal crops * Foliar application * Leaf spot diseases
00606008	Seed crops - Herbs, spices and medicinal crops * Foliar application * *Phoma*
10993200	Seed crops * Foliar application * Miscellaneous diseases
19993200	Herbs, spices and medicinal crops * Foliar application * Fungal diseases (1)
12653204	Plum trees * Foliar application * Brown rot(s) (*Monilia* sp.)
12653201	Plum trees * Foliar application * Rust(s)
17303203	Rose * Foliar application * Powdery mildew
17303211	Rose * Foliar application * Grey mould
01145004	Salsify * Foliar application * Brown leaf spot
16903201	Salsify * Foliar application * Powdery mildew
16903202	Salsify * Foliar application * Rust(s)

Use no.[69]	**Title**
00125012	Rye * Foliar application * Ear disease (*Microdochium* sp.)
00125011	Rye * Foliar application * Ear disease (*Fusarium* sp.)
00125016	Rye * Foliar application * Powdery mildew
00125008	Rye * Foliar application * Eyespot
15103232	Rye * Foliar application * Leaf blotch/scald
15103208	Rye * Foliar application * Rust(s)
15101212	Rye * Seed treatment * Fungi not in the Pythiaceae family
15801201	Soya bean * Seed treatment * Fungi not in the Pythiaceae family
15853205	Tobacco * Foliar application * Grey mould
15853204	Tobacco * Foliar application * *Sclerotinia*
16953206	Tomato * Foliar application * Powdery mildew
16953203	Tomato * Foliar application * Grey mould and *Sclerotinia* diseases
16952206	Tomato * Soil treatment * Fungi not in the Pythiaceae family
16952501	Tomato * Soil treatment * Nematodes
15903204	Sunflower * Foliar application * *Phoma*
15903203	Sunflower * Foliar application * *Phomopsis*
12703206	Grapevine * Foliar application * Black rot
12703204	Grapevine * Foliar application * Powdery mildew
12703205	Grapevine * Foliar application * Grey mould

Annex 4: Summary of toxicological parameters for the active substances in the SDHI class

List of ASs in the SDHI class (source: FRAC)	Harmonised toxicological classification (ATP no.)	Proposed toxicological classification, EFSA conclusion	Carcinogenicity* EFSA and/or ECHA (RAC) or Standing Committee on the Food Chain and Animal Health (for boscalid) conclusion	Source of the toxicity reference values	ADI (mg/kg bw/day)	ARfD (mg/kg bw)	AOEL (mg/kg bw/day)	ADME (% absorption, distribution, metabolism and excretion of major metabolites in rats)
Benzovindiflupyr	Acute Tox. 3, H301 Acute Tox. 3, H331 (ATP09, 2016)	Acute Tox. 3, H301 Acute Tox. 3, H331 EFSA, 2015	Increased incidence of thyroid follicular cell adenomas in male rats. Underlying mode of action (MoA): UDPGT (uridine 5'-diphospho-glucuronyltransferase) enzymatic induction via activation of the CAR nuclear receptor resulting in increased clearance of thyroid hormones and a compensatory increase in TSH responsible for the proliferation of follicular cells - considered adequately supported by dedicated mechanistic studies. MoA considered as not relevant to humans (Paragraph 3.9.2.5.3 of the CLP Guidance Document) → no classification Increased incidence of Harderian gland adenomas in mice at all doses. In the absence of dose-response relationship, pre-neoplastic lesions and carcinomas, in the absence of increased incidence of Harderian gland tumours in rats, and because this structure does not exist in humans due to the lack of a nictitating membrane (Paragraph 3.9.2.3.2	EFSA, 2015	0.05	0.1	0.04	Total rapid oral absorption at low doses but incomplete (60%) oral absorption at higher doses (40 mg/kg bw) Wide distribution (liver, kidneys) No accumulation Rapid excretion (mainly via bile) Very extensive metabolism by demethylation, hydroxylation and conjugation

List of ASs in the SDHI class (source: FRAC)	Harmonised toxicological classification (ATP no.)	Proposed toxicological classification, EFSA conclusion	Carcinogenicity* EFSA and/or ECHA (RAC) or Standing Committee on the Food Chain and Animal Health (for boscalid) conclusion	Source of the toxicity reference values	ADI (mg/kg bw/day)	ARfD (mg/kg bw)	AOEL (mg/kg bw/day)	ADME (% absorption, distribution, metabolism and excretion of major metabolites in rats)
			of the CLP Guidance Document) → no classification RAC Opinion of 04/12/2014 CLH-O-0000001426-86-28/F					
Bixafen	No notification	NC EFSA, 2012	No carcinogenic potential in rats and mice EFSA, 2012	EFSA, 2012	0.02	0.2	0.13	Rapid and extensive absorption (85%) Wide distribution (liver and kidneys) No accumulation Rapid excretion (mainly via bile) Metabolism by demethylation, hydroxylation and conjugation (glucuronic acid and glutathione) and marginally via cleavage of the amide bridge
Boscalid	No notification	NC, Review report, Standing Committee on the Food Chain and Animal Health, EC 2008 (assessment not seen by EFSA)	Increased incidence of thyroid follicular cell adenomas in male rats. Underlying mode of action: UDPGT (uridine 5'-diphospho-glucuronyltransferase) enzymatic induction supported by mechanistic studies. MoA considered as not relevant to humans (Paragraph 3.9.2.5.3 of the CLP Guidance Document) → no proposed classification Review report, Standing Committee on the Food Chain and Animal Health, EC 2008	Standing Committee on the Food Chain and Animal Health, EC 2008 Under re-assessment at EU level	0.04	No	0.1	Rapid but incomplete (44%) oral absorption Wide distribution (liver and adipose tissue and to a lesser extent kidneys and thyroid) No accumulation Rapid excretion (mainly via bile) Very extensive metabolism by hydroxylation of the diphenyl ring and glucuronidation

List of ASs in the SDHI class (source: FRAC)	**Harmonised toxicological classification (ATP no.)**	**Proposed toxicological classification, EFSA conclusion**	**Carcinogenicity*** **EFSA and/or ECHA (RAC) or Standing Committee on the Food Chain and Animal Health (for boscalid) conclusion**	**Source of the toxicity reference values**	**ADI (mg/kg bw/day)**	**ARfD (mg/kg bw)**	**AOEL (mg/kg bw/day)**	**ADME (% absorption, distribution, metabolism and excretion of major metabolites in rats)**
Carboxin	Skin Sens. 1B, H317 STOT RE 2, H373 RAC Opinion, 05/12/2017	Skin Sens. 1B, H317 Carc. 2, H351 EFSA, 2010	Increased incidence of hepatocellular carcinomas in male rats and increased incidence and early onset of lung adenomas in male mice. → proposed classification: carcinogenic Cat. 2, H351 EFSA, 2010 Increased hepatocellular carcinomas only in males at a dose exceeding the maximum tolerated dose (high mortality). The incidence of lung tumours (adenomas and adenocarcinomas combined) does not exceed the historical control data → no classification RAC Opinion of 04/12/2014 CLH-O-0000001412-86-180/F	EFSA, 2010	0.008	Not necessary	0.055	Rapid and extensive (80%) absorption Widespread distribution No accumulation Rapid and extensive excretion (mainly via urine) Metabolism by oxidation, hydroxylation, cleavage of the amide bridge and glucuronidation

List of ASs in the SDHI class (source: FRAC)	Harmonised toxicological classification (ATP no.)	Proposed toxicological classification, EFSA conclusion	Carcinogenicity* EFSA and/or ECHA (RAC) or Standing Committee on the Food Chain and Animal Health (for boscalid) conclusion	Source of the toxicity reference values	ADI (mg/kg bw/day)	ARfD (mg/kg bw)	AOEL (mg/kg bw/day)	ADME (% absorption, distribution, metabolism and excretion of major metabolites in rats)
Fluopyram	NC (ATP09, 2016)	Carc. 2, H351 EFSA, 2013	Increased incidence of hepatocellular adenomas and carcinomas in female rats and increased incidence of thyroid follicular cell adenomas in male mice. → proposed classification: carcinogenic Cat. 2, H351 EFSA, 2013 Mode of action underlying liver tumours in rats and thyroid tumours in mice: UDPGT (uridine 5'-diphospho-glucuronyltransferase) enzymatic induction via activation of the CAR nuclear receptor - considered adequately supported by dedicated mechanistic studies. MoA considered as not relevant to humans. → no classification RAC Opinion of 04/12/2014 CLH-O-0000001412-86-46/F	EFSA, 2013	0.012	0.5	0.05	Rapid and extensive (93%) absorption, entero-hepatic cycle Wide distribution (liver, kidneys, and to a lesser extent, erythrocytes, adrenals, thyroid and ovaries) Low potential for accumulation Near-complete excretion after 168 hours (via urine and bile) Extensive metabolism (hydroxylation, oxidation, cleavage and conjugation)
Flutolanil	Intention (NL): NC Submission date 09/2018	NC EFSA, 2008	No carcinogenic potential in rats and mice EFSA, 2008	EFSA, 2008	0.09	Not necessary	0.56	Rapid but incomplete (70%) absorption Wide distribution No accumulation Rapid and extensive excretion (mainly via urine) Extensive metabolism by depropylation, hydroxylation and conjugation

List of ASs in the SDHI class (source: FRAC)	**Harmonised toxicological classification (ATP no.)**	**Proposed toxicological classification, EFSA conclusion**	**Carcinogenicity*** **EFSA and/or ECHA (RAC) or Standing Committee on the Food Chain and Animal Health (for boscalid) conclusion**	**Source of the toxicity reference values**	**ADI (mg/kg bw/day)**	**ARfD (mg/kg bw)**	**AOEL (mg/kg bw/day)**	**ADME (% absorption, distribution, metabolism and excretion of major metabolites in rats)**
Fluxapyroxad	Intention (UK): NC Public consultation of 05/2018	Carc. 2, H351 EFSA, 2012	Increased incidence of hepatocellular adenomas (males and females) and carcinomas (males) in rats and increased incidence of thyroid follicular cell adenomas and carcinomas in male rats. → proposed classification: carcinogenic Cat.2, H351 EFSA, 2012	EFSA, 2012	0.02	0.25	0.04	Rapid but incomplete (68%) oral absorption Wide distribution (liver, adipose tissue and adrenals) Low potential for accumulation Near-complete excretion within three days, mainly via bile Very extensive metabolism mainly by hydroxylation of the diphenyl ring, loss of a fluorine atom, N-demethylation and conjugation, and marginally by cleavage of the amide bridge
Isofetamid	No notification	NC EFSA, 2015	No carcinogenic potential in rats and mice EFSA, 2015	EFSA, 2015	0.02	1	0.05	Rapid and ample (> 80%) absorption Wide distribution No accumulation Complete excretion within 48 hours mainly via bile Very extensive (> 80%) metabolism by O-dealkylation, oxidation, hydroxylation and conjugation

List of ASs in the SDHI class (source: FRAC)	Harmonised toxicological classification (ATP no.)	Proposed toxicological classification, EFSA conclusion	Carcinogenicity* EFSA and/or ECHA (RAC) or Standing Committee on the Food Chain and Animal Health (for boscalid) conclusion	Source of the toxicity reference values	ADI (mg/kg bw/day)	ARfD (mg/kg bw)	AOEL (mg/kg bw/day)	ADME (% absorption, distribution, metabolism and excretion of major metabolites in rats)
Isopyrazam	Intention (UK): Acute Tox. 4, H302 Skin Sens. 1, H317 Carc. 2, H351 Repr. 2, H361d Submission date 09/2018	Acute Tox. 4, H302 Skin Sens. 1, H317 Carc. 2, H351 Repr. 2, H361d EFSA, 2012	In rats, increased incidence of hepatocellular adenomas and uterine adenocarcinomas. → proposed classification: carcinogenic Cat.2, H351 EFSA, 2012	EFSA, 2012	0.03	0.2	0.05	Rapid but incomplete (64%) oral absorption Wide distribution (liver, kidneys and adrenals) Low potential for accumulation Near-complete excretion within two days following a single administration, slower excretion after repeated administration, mainly via bile Very extensive metabolism mainly by hydroxylation in the bicyclo-isopropyl moiety, oxidation, N-demethylation and conjugation
Penflufen	Carc. 2, H351 RAC Opinion, 15/10/2018	Carc. 2, H351 EFSA, 2012	In rats, increased incidence of hepatocellular and ovarian adenomas in females and histiocytic sarcomas and astrocytomas in males. In mice, increase in hepatocellular adenomas (males and females) and hepatic adenocarcinomas in males. Mode of action underlying liver tumours by activation of the CAR/PXR nuclear receptors supported by mechanistic data. The mode(s) of action underlying other tumours is/are not established, but their incidence is not statistically significant and is marginally increased compared to the respective historical control	EFSA, 2012	0.04	0.5	0.077	Rapid and complete oral absorption Wide distribution (liver, erythrocytes, kidneys, adrenals and adipose tissue) Low potential for accumulation Rapid excretion via bile and urine Very extensive metabolism mainly by hydroxylation, N-methylation, oxidation and conjugation

List of ASs in the SDHI class (source: FRAC)	Harmonised toxicological classification (ATP no.)	Proposed toxicological classification, EFSA conclusion	Carcinogenicity* EFSA and/or ECHA (RAC) or Standing Committee on the Food Chain and Animal Health (for boscalid) conclusion	Source of the toxicity reference values	ADI (mg/kg bw/day)	ARfD (mg/kg bw)	AOEL (mg/kg bw/day)	ADME (% absorption, distribution, metabolism and excretion of major metabolites in rats)
			data. → Classification: carcinogenic Cat. 2, H351 RAC Opinion of 15/10/2018 CLH-O-0000001412-86-233/F					
Penthiopyrad	NC (ATP10, 01/12/2018)	Carc. 2, H351 EFSA, 2013	In rats, increased incidence of thyroid follicular cell adenomas in males. In mice, increase in hepatocellular adenomas in males. → proposed classification: carcinogenic Cat. 2, H351 EFSA, 2013 Mode of action underlying thyroid tumours in rats and liver tumours in mice via activation of the CAR nuclear receptor considered adequately supported by the available mechanistic studies. MoA considered as not relevant to humans. → no classification RAC Opinion of 04/12/2015 CLH-O-0000001412-86-78/F	EFSA, 2013	0.1	0.75	0.1	Rapid and extensive (> 83%) absorption Wide and rapid distribution No accumulation Rapid excretion (> 95% within 24 hours) mainly via bile Very extensive metabolism by N-demethylation and oxidation

List of ASs in the SDHI class (source: FRAC)	**Harmonised toxicological classification (ATP no.)**	**Proposed toxicological classification, EFSA conclusion**	**Carcinogenicity*** **EFSA and/or ECHA (RAC) or Standing Committee on the Food Chain and Animal Health (for boscalid) conclusion**	**Source of the toxicity reference values**	**ADI (mg/kg bw/day)**	**ARfD (mg/kg bw)**	**AOEL (mg/kg bw/day)**	**ADME (% absorption, distribution, metabolism and excretion of major metabolites in rats)**
Sedaxane	Intention (FR): Carc. 2, H351 Public consultation of 03/08/2018	Carc. 2, H351 EFSA, 2013	In rats, increased incidence of hepatocellular and thyroid follicular cell adenomas in males and uterine adenocarcinomas in females. In mice, increase in hepatocellular adenomas and carcinomas in males. → proposed classification: carcinogenic Cat. 2, H351 EFSA, 2013 Mechanistic data provided in the classification dossier (CLH report) support a mode of action via activation of the CAR/PXR receptors for liver and thyroid tumours. For uterine tumours, the MoA is considered inadequately supported to rule out their relevance to humans. → proposed classification: carcinogenic Cat. 2, H351 (CLH report under public consultation)	EFSA, 2013	0.11	0.3	0.28	Rapid and extensive (> 80%) oral absorption Wides distribution (liver and kidneys) No accumulation Rapid excretion mainly via bile Very extensive metabolism mainly by demethylation, hydroxylation, oxidation and conjugation

List of ASs in the SDHI class (source: FRAC)	Harmonised toxicological classification (ATP no.)	Proposed toxicological classification, EFSA conclusion	Carcinogenicity* EFSA and/or ECHA (RAC) or Standing Committee on the Food Chain and Animal Health (for boscalid) conclusion	Source of the toxicity reference values	ADI (mg/kg bw/day)	ARfD (mg/kg bw)	AOEL (mg/kg bw/day)	ADME (% absorption, distribution, metabolism and excretion of major metabolites in rats)
Pydiflumetofen	Intention (FR): NC Public consultation of 03/06/2018	NC EFSA, 2018 (in press)	Increased incidence of hepatocellular adenomas and adenocarcinomas in male mice. Mode of action underlying liver tumours by activation of the CAR/PXR nuclear receptors supported by mechanistic data → no classification However, a lack of data enabling a conclusion to be drawn as to the potential adverse effects of succinate dehydrogenase inhibition in humans is underlined. EFSA, 2018	EFSA, 2018	0.09	0.3	0.1	Rapid oral absorption (> 85%) Wide distribution (liver and kidneys) No accumulation Rapid excretion mainly via bile Very extensive metabolism via cleavage of the molecule forming the major metabolite TCP (trichlorophenol) as well as pyrazole metabolites. Other metabolic pathways: demethylation, hydroxylation, oxidation and conjugation

NC: Not classified
H301: Toxic if swallowed
H302: Harmful if swallowed
H317: May cause an allergic skin reaction
H331: Toxic if inhaled
H373: May cause damage to organs through prolonged or repeated exposure
H351: Suspected of causing cancer
H361: Suspected of damaging fertility or the unborn child

NA: Not applicable
NE: Not evaluated

*: The harmonised classification of a chemical according to Regulation (EC) No 1272/2008 is the responsibility of ECHA. However, a proposal to classify a phytopharmaceutical substance is reported in EFSA's conclusions following its assessment according to Regulation (EC) No 1107/2009. This column contains explanations of the two previous columns.

ECHA: European Chemicals Agency
EFSA: European Food Safety Authority
RAC: Committee for Risk Assessment (the ECHA committee in charge of preparing opinions on proposals for harmonised classification)
EC: European Commission

Annex 5: Summary of ecotoxicological parameters for the active substances in the SDHI class

List of ASs in the SDHI class (source: FRAC)	Reference	Maximum DT50 in soil [days] Mean DT50 in soil [days, value normalised to 20°C and pF2] Characterisation of persistence in soil	DT50 in water [days]	P, B, T criteria	Acute toxicity to birds [mg/kg bw/day]	Chronic toxicity to birds [mg/kg bw]	Acute toxicity to mammals [mg/kg bw/day]	Chronic toxicity to mammals [mg/kg bw]	Toxicity to aquatic organisms PNEC [µg as/L]	Ecotoxicological classification	Chronic toxicity to soil organisms Laboratory study PNEC [mg as/kg d.w.soil]	Oral toxicity to bees Laboratory study [µg/bee]	Chronic toxicity to bees Laboratory study [µg/bee]
Benzovindiflupyr	EFSA Journal 2015;13(3):4043	1000 (Lab) 184 (Field) Very high	27.1	P,T	1315	25	55	6.8	0.035 (fish)	H400 H410 ATP9 Reg. (EU) 1272/2008	1.562	>109	>100
Bixafen	EFSA Journal 2012;10(11):2917	1235 (Field, biphasic) 203.2 (Field) Very high	26.4	P,T	>2000	24.5	>5000	33.3	0.46 (fish)	H400 H410 ANSES in accordance with Reg. 1272/2008	20	>100	>121.4
Boscalid	Review report SANCO/3919 /2007-rev. 5 21 January 2008	208 (Field) 232 (Lab)	5.2	P	>2000	24.1	>5000	67	12.5 (fish)	H411 ANSES in accordance with Reg. 1272/2008	0.24	>166	>200
Carboxin	EFSA Journal 2010;8(10):1857	11 (Field) 0.28 (Laboratory) Very low to low	13.6		>2150	83	2588	20	23 (fish)	H400 H410 ANSES in accordance with Reg. 1272/2008 (RAC43-ECHA)	10	>100	>100
Fluopyram	EFSA Journal 2013;11(4):3052	347 (Field, biphasic) 123.1 (Field, median) High to very high	19.8	P	>2000	4.5	>2000	14.5	13.5 (fish)	H411 ATP9 Reg. (EU) 1272/2008	2.284	>102.3	>100
Flutolanil	EFSA Scientific Report (2008) 126, 1-63	412 (Laboratory) 190 (Laboratory) High to very high	42	P	>2000	247	>10000	157	23.3 (fish)	H411 ANSES in accordance with Reg. 1272/2008	2.58	>208.7	>200

List of ASs in the SDHI class (source: FRAC)	Reference	Maximum DT50 in soil [days] Mean DT50 in soil [days, value normalised to 20°C and pF2] Characterisation of persistence in soil	DT50 in water [days]	P, B, T criteria	Acute toxicity to birds [mg/kg bw/day]	Chronic toxicity to birds [mg/kg bw]	Acute toxicity to mammals [mg/kg bw/day]	Chronic toxicity to mammals [mg/kg bw]	Toxicity to aquatic organisms PNEC [µg as/L]	Ecotoxicological classification	Chronic toxicity to soil organisms Laboratory study PNEC [mg as/kg d.w.soil]	Oral toxicity to bees Laboratory study [µg/bee]	Chronic toxicity to bees Laboratory study [µg/bee]
Fluxapyroxad	EFSA Journal 2012;10(1):2522	370 (Field, biphasic) 151 (Field) Medium to very high	4.1	P	>2000	33.6	>2000	10	2.9 (fish)	H400 H410 ANSES in accordance with Reg. 1272/2008	0.598	>110.39	>100
Isofetamid	EFSA Journal 2015;13(10):4265	55 (Laboratory) 37.1 (Laboratory) Moderate	18.1	P	>2000	25	>2000	57.1	18 (fish)	H411 ANSES in accordance with Reg. 1272/2008	2.074	>30	>100
Isopyrazam	EFSA Journal 2012;10(3):2600	629 (Field) 84 (Field) Medium to very high	2.3	P,T	>2000	32.5	>2000	41	0.258 (fish)	H400 H410 ANSES in accordance with Reg. 1272/2008	12	>95.5	>100
Penthiopyrad	EFSA Journal 2013;11(2):3111	406 (Laboratory) 121.5 (Laboratory) Medium to very high	9.9	P	>2250	206.8	>2000	54	2.9 (fish)	H400 H410 ATP10 Reg. (EU) 1272/2008	9.6	>500	>500
Sedaxane	EFSA Journal 2013;11(1):3057	438 (Field) 100 (Field) Moderate to medium	17.3	P	>2000	96.3	2000	103.8	6.2 (fish)	H400 H410 ANSES in accordance with Reg. 1272/2008	0.28	>4	>100
Pydiflumetofen	EFSA Journal 2018 (in press)	8540 (Field) 1334 (Field) Very high	16.2	P	>2000	90.1	>5000	36	2.5 (fish)	H400 H410 ANSES in accordance with Reg. 1272/2008	3.18	>116	>100

PBT criteria:
Persistence: An active substance, safener or synergist meets the criteria for persistence when:
- its half-life in fresh or estuarine water is higher than 40 days,
- its half-life in fresh or estuarine water sediment is higher than 120 days, or
- its half-life in soil is higher than 120 days.

Bioaccumulative if BCF > 2000
Toxic if NOEC or EC10 < 0.01/L for aquatic organisms

Annex 6: Analysis of publications on the ecotoxicity of SDHIs

In the first publication[72], the authors studied the effects of sedaxane (mortality, hatching, heart rate and expression of SDH-specific genes) on fish (zebrafish) embryos. They described effects dependent on the tested concentration. Nonetheless, the embryos were exposed to a concentration range from 1 to 10 mg/L (i.e. 1000 µg/L to 10,000 µg/L) for five days. These concentrations were 160 to 1600 times higher than the PNEC (6.2 µg/L) used for *a priori* risk assessments and were thus far above the expected concentrations in the environment and those deemed to be toxic. They did not correspond to the predicted environmental concentrations in the assessment dossiers for this AS.

In the second publication by the same team[73], the authors studied the effects of isopyrazam (mortality, hatching, heart rate and expression of SDH-specific genes) on fish (zebrafish) embryos. The embryos were exposed to a concentration range from 0.025 to 0.5 mg/L (i.e. 25 µg/L to 250 µg/L) for four days. These concentrations were 100 to 1000 times higher than the PNEC (0.258 µg/L) used for *a priori* risk assessments and were thus far above the expected concentrations in the environment. They did not correspond to the predicted environmental concentrations in the assessment dossiers for this AS.

In the third publication[74], insect neuronal cell lines were exposed to a series of pyrazole carboxamide compounds in an attempt to develop a substance with insecticidal properties. These substances were developed by removing the biphenyl ring and adding a diarylamine group. The authors indicate that one of the created substances may act as a potential insecticide after optimisation. The scope of this work is currently limited since none of the tested substances are known or used.

Lastly, in the fourth publication[75], the authors exposed *Xenopus* embryos to strobilurin and SDHI fungicides. Toxicity was studied following exposure to a single compound or a mixture of compounds. In this study, strobilurins were identified as more toxic than SDHIs (lethal doses in the range of µg/L and mg/L respectively). The authors indicate that malformations and dose-response relationships were more significant when the *Xenopus* embryos were exposed to mixtures of compounds.

72 Yao H, Yu J, Zhou Y, Xiang Q, Xu C. The embryonic developmental effect of sedaxane on zebrafish (Danio rerio). Chemosphere. 197 (2018) 299-305

73 Yao H, Xu X, Zhou Y, Xu C. Impacts of isopyrazam exposure on the development of early-life zebrafish (Danio rerio). Environ Sci Pollut Res Int. 25 (2018) 23799-23808

74 Ren Y, Yang N, Yue Y, Jin H, Tao K, Hou T. Investigation of novel pyrazole carboxamides as new apoptosis inducers on neuronal cells in Helicoverpa zea. Bioorg Med Chem. 26 (2018) 2280-2286

75 Wu S. et al. Single and mixture toxicity of strobilurin and SDHI fungicides to Xenopus tropicalis embryos. Ecotoxicol Environ Saf. 153 (2018) 8-15

Annex 7: Data on the SDHI resistance of pathogenic fungi

SDHIs have been used for over 40 years, and the first cases of resistance were limited to a few basidiomycetes due to the small number of treated crops (white rust of chrysanthemum and barley rust). Today, numerous pathogens are resistant (mutations in various SDH subunits (A, B, C or D) with a risk of resistance considered as moderate. Cross-resistance is non-systematic or incomplete[76,77]. Other mechanisms of SDHI resistance involve phenomena of metabolism or active efflux from compounds outside of the target cells[78,79] or mechanisms yet to be determined[80].

SDH therefore contains a major flavoprotein subunit that covalently binds to FAD, an iron-sulphur protein, as well as two subunits (C and D) that anchor the catalytic subunits A and B in the mitochondrial inner membrane. Subunits C and D only slightly bind to the heme ring (subunit B). In humans, genetic abnormalities (mutations or deletions in germ cells) in the various subunits and/or epimutations within gene promoter regions, leading to enzymatic alterations, are correlated with cancer transformation and progression[81,82].

All crop protection SDHIs target the ubiquinone-binding pocket, structurally defined as the interface between subunits C, D and B. Although a few amino acid residues important in the catalysis of ubiquinone reduction are strictly conserved across species, most amino acid residues of subunits C and D display a high degree of variation across species, hence the remarkable structural variability of SDHIs. Primary sequence conservation for subunits C and D is therefore low[83].

Some cases of resistance with mutations have been reported in numerous scientific publications and on the FRAC website. Some of these mutations affect the shape of the SDHI binding pocket (as in *Z. tritici, B. cinerea* and several ascomyetes) while others have an impact on subunits C and D. They appear to be involved in iron chelation (heme ring) as in *Alternaria.* Some ubiquinone site residues (subunits A and B) which are key for SDHI binding have been found mutated in resistant laboratory and field isolates. The various mutations involved in resistance can have a temporary effect on the fitness of strains[76]. The mutations responsible for resistance lead to resistance factors of 20 to 100, often related to mutations in the various subunits. More than 27 mutations can confer SDH resistance in selected field pathogens. These mutations provide an advantage in terms of resistance and for the functioning of ubiquinone binding and enzymatic catalysis. In these cases, the selected mutations contribute to the proper functioning of the target enzyme.

However, it seems that numerous cases of strong resistance are not related to mutations in SDH subunits. This is the case, for example, of *Z. tritici* strains, which display strong resistance to fluopyram and isofetamid. Mechanisms such as ABC transporters (multidrug resistance (MDR) transporters) and increased metabolism are suspected[78].

Due to resistance in numerous target pathogens of SDHIs, it is for example recommended to limit their use on field crops and grapevines to two applications, including one annual treatment per chemical group[84].

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76 Sierotzki H, Scalliet G. A review of current knowledge of resistance aspects for the next-generation succinate dehydrogenase inhibitor fungicides. Phytopathology 103 (2013) 880–887

77 Scaliet G. et al. Mutagenesis and functional studies with succinate dehydrogenase inhibitors in the wheat pathogen Mycosphaerella graminicola. PLoS One. 7 (2012) e35429

78 Yamashita M., Fraaije B. Non-target site SDHI resistance is present as standing genetic variation in field populations of Zymoseptoria tritici. Pest Manag Sci. 74 (2018) 672-681

79 Sang H, Hulvey J, Popko JT Jr, Lopes J, Swaminathan A, Chang T, Jung G. A pleiotropic drug resistance transporter is involved in reduced sensitivity to multiple fungicide classes in Sclerotinia homoeocarpa (F.T. Bennett). Mol Plant Pathol. 16 (2015) 251-61

80 Menzies J., Mc Leod R., Tosi L., Cappelli C. Occurrence of a carboxin-resistant strain of Ustilago nuda in Italy. Phytopathol. Mediterr. 44 (2005) 216-219

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82 Gill AJ. Succinate Dehydrogenase (SDH)-deficient Neoplasia Histopathology 72 (2018) 106–116

83 Cecchini G. Function and structure of complex II of the respiratory chain. Annu Rev Biochem. 72 (2003) 77-109

84 http://www.vignevin.com/fileadmin/users/ifv/2015_New_Site/Home_page/Fichiers/2018/Note_technique_commune_Vigne_2018.pdf

The SDHI doses capable of limiting the growth of sensitive pathogens vary depending on the compound, and the most effective is generally chosen for a given crop and for identified pests. Some examples of IC_{50} (dose with 50% growth inhibition) values obtained with various compounds show doses ranging from 0.02 to 1 mg/L (around 0.06 to 3 µM) depending on the compound and pathogen. Note that these doses inhibit whole micro-organisms and not cellular or enzymatic extracts, suggesting that the doses reaching the target enzyme are much lower (need to cross through fungal walls and cell membranes) (table below).

Table: Examples of SDHI concentrations (mg/L) inhibiting some crop pathogens by 50%.

Name	Fluopyram	Boscalid	Isofetamid	Bixafen	Fluxapyroxad	Benzovindiflupyr	Penthiopyrad
Z. tritici[78,85]	0.51	-	0.16	0.073	0.10; 0.02		
E. necator[86]		≤ 1					
Alternaria alternata[87]	0.97	0.16					
S. sclerotiorum[88]		0.38-0.395					
C. acutatum, C. cereale[89]					≤ 0.1	≤ 0.1	≤ 0.1
P. italicum		0.15					

85 Gutiérrez-Alonso O, Hawkins NJ, Cools HJ, Shaw MW, Fraaije BA. Dose-dependent selection drives lineage replacement during the experimental evolution of SDHI fungicide resistance in *Zymoseptoria tritici*. Evol Appl. 10 (2017) 1055-1066

86 Cherrad S., Charnay A., Hernandez C. Steva H., Belbahri L., Vacher S. Emergence of boscalid-resistant strains of Erysiphe necator in French vineyards. Microbiological Research 216 (2018) 79-84

87 Avenot H., Michailides T.J. Progress in understanding molecular mechanisms and evolution of resistance to succinate dehydrogenase inhibiting (SDHI) fungicides in phytopathogenic fungi. Crop Prot 29 (2010) 643-51

88 Hu S., Zhang J ., Zhang Y., He S., Zhu F. Baseline sensitivity and toxic actions of boscalid against Sclerotinia sclerotiorum. Crop Prot 110 (2018) 83-90

89 Ishii H, Zhen F, Hu M, Li X, Schnabel G. Efficacy of SDHI fungicides, including benzovindiflupyr, against Colletotrichum species. Pest Manag Sci. 72 (2016) 1844-53