TRABAJO ORIGINAL

Succinate dehydrogenase inhibitors fungicides: a review of their impact on human health and epigenetic changes.

Fungicidas inibidores da succinato desidrogenase: uma revisão de seu impacto na saúde humana e alterações epigenéticas.

Fungicidas inhibidores de la succinato deshidrogenasa: una revisión de su impacto en la salud humana y los cambios epigenéticos.

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Received: March 15, 2023
Accepted: November 18, 2023

Editors: Edda Villaamil Lepori and Adriana Ridolfi

Abstract. Succinate dehydrogenase inhibitors (SDHIs), fungicides currently most used in agriculture in Brazil, act by blocking the enzyme succinate dehydrogenase (SDH) from plant pathogens. However, studies show that SDHIs can not only inhibit SDH activity in target fungi, but also block that activity in human cells. Considering the medical and agricultural implications of SDH, the purpose of this narrative review is to describe the relationship between exposure to fungicides SDHIs and epigenetic regulation of SDH associated with the development of gastrointestinal stromal tumor, pheochromocytoma/paraganglioma, and cancer. The results obtained with the research showed that the human SDH enzyme exhibited sensitivity to some tested SDHIs, which may cause microcephaly and defects in neurological development. Deficiency of SDH activity causes accumulation of succinate which can act as an oncometabolite inhibiting iron-dependent dioxygenases and alpha-ketoglutarate, eleven translocation -TET and histone demethylases, inducing epigenetic changes that lead to multiple cancers and other diseases. Therefore, further *in vitro* and *in vivo* analyzes should be performed to assess susceptibility to diseases influenced by the toxic effect of SDHIs.

Keywords: SDHI; Agriculture; DNA methylation; Cancer; Paraganglioma; Gastrointestinal stromal tumor.

Resumo. Os inibidores da succinato desidrogenase (SDHIs), fungicidas atualmente mais utilizados na agricultura no Brasil, atuam bloqueando a enzima succinato desidrogenase (SDH) de fitopatógenos. No entanto, estudos mostram que SDHIs podem não apenas inibir a atividade de SDH em fungos alvo, mas também bloquear essa atividade em células humanas. Considerando as implicações médicas e agrícolas do SDH, o objetivo desta revisão narrativa é descrever a relação entre a exposição a fungicidas SDHIs e a regulação epigenética do SDH associada ao desenvolvimento de tumor estromal gastrointestinal, feocromocitoma/paraganglioma e câncer. Os resultados obtidos com a pesquisa mostraram que a enzima SDH humana apresentou sensibilidade a alguns SDHIs testados, que podem causar microcefalia e defeitos no desenvolvimento neurológico. A deficiência da atividade da SDH causa acúmulo de succinato que pode atuar como um oncometabólito inibindo as dioxigenases dependentes de ferro e alfa-cetoglutarato, onze translocações -TET e histonas desmetilases, induzindo alterações epigenéticas que levam a múltiplos cânceres e outras doenças. Portanto, análises adicionais *in vitro* e *in vivo* devem ser realizadas para avaliar a suscetibilidade a doenças influenciadas pelo efeito tóxico dos SDHIs.

Palavras-chave: SDHI; Agricultura; Metilação do DNA; Câncer; Paraganglioma; Tumor estromal gastrointestinal.

Resumen. Los inhibidores de la succinato deshidrogenasa (SDHI), los fungicidas actualmente más utilizados en la agricultura en Brasil, actúan bloqueando la enzima succinato deshidrogenasa (SDH) de los patógenos de las plantas. Sin embargo, los estudios muestran que los SDHI no solo pueden inhibir la actividad de SDH en los hongos objetivo, sino que también bloquean esa actividad en las células humanas. Teniendo en cuenta las implicaciones médicas y agrícolas de SDH, el propósito de esta revisión narrativa es describir la relación entre la exposición a fungicidas SDHI y la regulación epigenética de SDH asociada con el desarrollo de tumores del estroma gastrointestinal, feocromocitoma/paraganglioma y cáncer. Los resultados obtenidos con la investigación mostraron que la enzima SDH humana mostró sensibilidad a algunos SDHI probados, lo que puede causar microcefalia y defectos en el desarrollo neurológico. La deficiencia de la actividad de SDH provoca la acumulación de succinato que puede actuar como un oncometabolito que inhibe las dioxigenasas dependientes de hierro y el alfa-cetoglutarato, once translocaciones -TET e histona desmetilasas, induciendo cambios epigenéticos que conducen a múltiples cánceres y otras enfermedades. Por lo tanto, se deben realizar más análisis *in vitro* e *in vivo* para evaluar la susceptibilidad a enfermedades influenciadas por el efecto tóxico de los SDHI.

Palabras clave: SDHI; Agricultura; Metilación del ADN; Cáncer; Paraganglioma; Tumor del estroma gastrointestinal.

INTRODUCTION

SDH is of agricultural importance, as many fungicides have been and/or will be designed specifically targeting this enzyme in plant fungal pathogens (Moosavi *et al.* 2020). SDHI fungicides are inhibitors of mitochondrial respiration, which act by blocking the pathogen's succinate dehydrogenase, interruptingelectron flow and respiration (Tian *et al.* 2020).

Aerobic respiration is a biological process that constitutes the main source of energy for living organisms, since mitochondrial ATP synthesis depends on the ETC, which couples the generation of an electrochemical gradient to the oxidation of NADH. and FADH2 and the reduction of oxygen to water. ETC is composed of four complexes and two mobile electron carriers (coenzyme Q and cytochrome c) (Van Vranken *et al.* 2015).

Three cellular processes are involved in aerobic respiration: glycolysis, the TCA, and OXPHOS (Moosavi *et al.* 2020). Electrons derived from the oxidation of NADH by complex I or TCA succinate by the enzyme succinate dehydrogenase, also known as complex II (CII) of ETC or succinate: ubiquinone oxidoreductase (SQR) (Moosavi *et al.* 2020), are passed along the ETC, along with pumping protons and establishing the proton gradient across the inner mitochondrial membrane. Ultimately, the controlled flow of protons in this electrochemical gradient is utilized by complex V (ATP synthase) to catalyze ATP synthesis (Van Vranken *et al.* 2015).

The normal enzyme activity of SDH serves to suppress tumors in humans (Van Vranken *et al.* 2015). However, mutation that results in loss of function in any of the four subunits (SDHA, SDHB, SDHC, and SDHD), destabilizes the SDH protein complex and eliminates its enzymatic activity (Zhao *et al.* 2020).

SDH expression is mainly regulated through genetic and epigenetic mechanisms, while biochemical factors mainly regulate the activity of this enzyme (Moosavi *et al.* 2020). The loss or decrease of SDH activity leads to the accumulation of succinate that induces epigenetic changes in cancer cells (Dalla Pozza *et al.* 2020).

Because of the universal role of SDH in cellular respiration and mitochondrial metabolism in living organisms, studies have shown that fungicides belonging to the chemical group of benzamides and carboxamides, succinate dehydrogenase inhibitors (SDHIs), have the potential to inhibit the human SDH enzyme (Bénit *et al.* 2019). Some studies have recently raised concerns about the safety of agrochemicals, particularly SDHI fungicides, which are widely used around the world to control fungi in various agricultural crops (Van Vranken and Rutter 2015; Bénit *et al.* 2019; Brenet *et al.* 2021).

According to the toxicological reclassification of pesticides by (Min. da Saúde 2022a), the toxicological and environmental class of the SDHIs found were: Category 2 -Highly Toxic Product - red band; Category 3 – Moderately Toxic Product – yellow band; Category 4 -Low Toxic Product – blue belt; and Category 5 -Product Unlikely to Cause Ac ute Injury – blue belt. The degree of toxicity of the substances that make up the SDHIs varies depending on the category (*Table 1*). Studies report the use of SDHIs in the main agricultural crops: almond, potato, tomato, grape, strawberry, apple, kiwi fruit, cucurbits, oil seed rape, cucumber, corn, barley, tulip bulbs, asparagus, wheat and barley (Sierotzki and Scalliet 2013).

Studies carried out by Collotta *et al.* (2013); Cao *et al.* (2019); Dalla Pozza *et al.* (2019) point out that fungicides can cause DNA mutations and affect gene expression through epigenetic mechanisms. Other studies developed by Van Vranken *et al.* (2015), Bénit *et al.* (2019), Brenet *et al.* (2021), because of the universal role of SDH in cellular respiration and mitochondrial metabolism in living organisms, expo-

sure to SDHI fungicides can cause adverse health outcomes in humans.

In this review, we integrate recent advances in the medical and agricultural implications of SDH, with the aim of describing the relationship between exposure to SDHI used in Brazil and the epigenetic regulation of SDH associated with the development of gastrointestinal stromal tumor, paraganglioma, and cancer.

MATERIAL AND METHODS

Literature revision

A search string search was performed using PubMed, with combinations of terms from the following categories: (a) *succinate dehydrogenase inhibitors*, *AND* and *OR* (b) *epigenetic regulation of succinate dehydrogenase*. The selection of references was based on studies that evaluated the consequences of SDHI fungicides in human health, and the epigenetic regulation of SDH associated with human diseases.

The work consisted of a bibliographic design, and the survey was limited to studies published from 2011 onwards. The searches were limited to the last ten years to allow the analysis of the works most current. The following were established as inclusion and exclusion criteria for selection: 1. Selection by the title criterion (relationship of the words that appear in the title with the subject of the review); 2. Selection criteria for the selected abstract (the selected study prioritizes the objective of the review); 3. Selection by full-text assessment for eligibility (methodology used is adequate, results relevant to the area); 4. Overview of selected articles (impact factor).

Additional references were included because they present relevant information on the classification of SDHIs and adverse results of agrochemicals to human health and the environment, through searches in the Sistema de Agrotóxicos e Fitossanitários (AGROFIT) of the Ministério da Agricultura, Pecuária e Abastecimento (MAPA), the Agência Nacional de Vigilância Sanitária (ANVISA), the Instituto Nacional de Câncer (INCA) and the Comitê Brasileiro de Ação a Resistência a Fungicidas (FRAC-BR).

This review summarizes the current state of knowledge about the risks of SDHI exposure in non-target organisms and the epigenetic regulation of SDH, drawing conclusions and making recommendations for future research. It is critical to note that review articles, like other types of scientific articles, are a type of research that uses bibliographic or electronic sources of information to obtain research results from other authors in order to theoretically support a specific topic (Botelho 2011).

Table 1. Classification of SDHI fungicides found in the review, based on the degree of toxicity of these substances, by the toxicological reclassification of pesticides by ANVISA (2019).

Common Name (Chemical Group): Product	Class Tox.	Hazard Class		
		Oral	Dermal	Inhalation
Fluxapyroxad (carboxamide): Adexar	2	Fatal if swallowed	Fatal in contact with skin	fatal if inhaled
Fluxapyroxad (carboxamide): Cuantiva; Veldara - Zoxamida (benzamide): Stimo WP; Zoxium 800 WP	3	Toxic if ingested	Toxic in contact with skin	Toxic if inhaled
Fluxapyroxad (carboxamide): Ativum; Audaz; Aumenax; Denaxo; Orkestra SC; Sesitra; Tivaro - Fluopyram (benzamide): Ilevo - Zoxamida (benzamide): Harpon WG; Stimo	4	Harmful if ingested	Harmful in contact with skin	Harmful if inhaled
Bixafen (carboxamide): Fox Xpro - Flutolanil (carboxamide): Helmet; Moncut - Fluxapyroxad (carboxamide): Blavity - Fluopicolide (benzamide): Infinito; Xavante - Fluopyram (benzamide): Valente Prime; Verango Prime	5	May be dangerous if ingested	May be dangerous in contact with skin	May be dangerous if inhaled

Adapted: Sistema de Agrotóxicos e Fitossanitários – AGROFIT. Brasil. Ministério da Agricultura, Pecuária e Abastecimento – MAPA (2022).

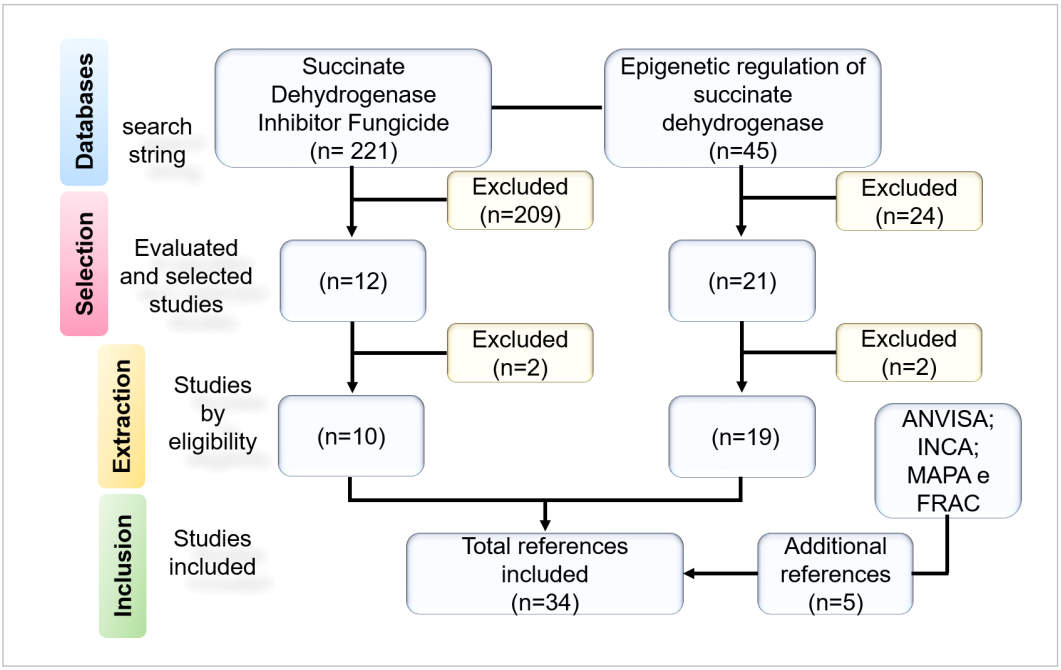

Figure 1. Flow diagram of the selection of studies in the period 2011-2021.

RESULTS

Selection of studies obtained from the research

The initial search resulted in 266 records, and four additional references were selected for detailed evaluation (*Figure 1*). After selection, 241 articles were excluded for the following reasons: 209 for not presenting any description of SDHIs associated with human disease (n = 12) and 24 for lack of data on epigenetic mechanisms involved in SDH regulation (n=21).

In the extraction stage, 10 selected articles on SDHI fungicides were included in the review (n=10) and two articles were excluded, and of the 21 articles on the epigenetic regulation of SDH, two articles were excluded because they did not specify the epigenetic mechanism involved in the development of human disease (n=19).

Of the 33 articles selected, 29 articles were eligible for inclusion because they included results related to the topic addressed, all written in English. In addition to the references obtained by the search string, publications from ANVISA, INCA, FRAC-BR and MAPA (Min. da Saúde 2022a; and 2022b; FRAC 2022; MAPA 2022) and one publication narrative review (Botelho 2011) were also included in this review. Finally, a total of 34 studies were included in the present analysis.

Classification of SDHIs according to ANVISA, INCA and MAPA

In the Sistema de Agrotóxicos e Fitossanitários - AGROFIT of MAPA (Min. da Saúde 2022b) for the chemical group (carboxamide), the following SDHIs were discovered: biixafem, flutolanil, and fluxapyroxad with formulation in concentrated suspension or emulsifiable concentrate, and fluopicolide, fluopyram, and zoxamide. benzamide chemical group in concentrated suspension, water-dispersible granules, and wettable powder.

The common name SDHI fungicide Bixafen classified in category 5 as a product unlikely to cause acute damage as determined by ANVISA, in the work developed by Bénit *et al.* (2019) was shown when glutamine is the major carbon source, the presence of SDHIs leads to time-dependent cell death. This process is significantly accelerated in fibroblasts derived from patients with neurological or neurodegenerative diseases due to RC impairment (encephalopathy originating from a partial SDH defect) and/or hypersensitivity to oxidative insults (Friedreich ataxia, familial Alzheimer's disease). On the other hand, Kamp *et al.* (2021) pointed out that fluxapyroxad SDHIs classified in categories 2, 3, 4 and 5 depending on the commercial product, did not result in changes in succinate or lactate levels after *in vivo* exposure in rats.

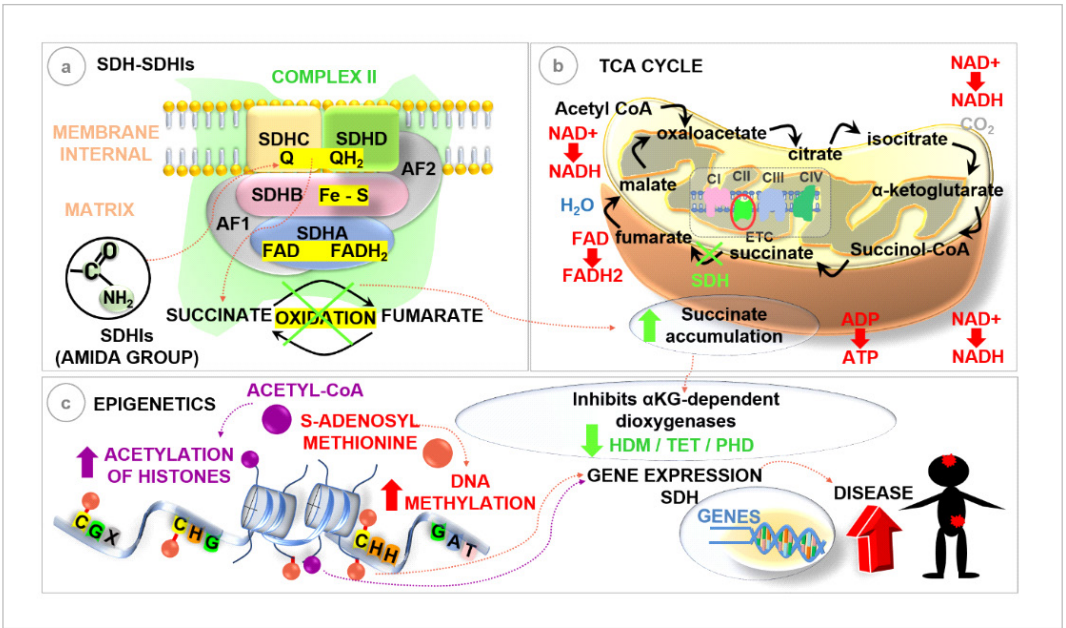

Figure 2. Schematic representation of succinate accumulation and epigenetic changes. (a) SDHIs bind to the SDH enzyme at the binding site of the ubiquinone "Q" subunits SDHB, -C and -D, blocking the oxidation of succinate. (b) Blockade of SDH enzymatic activity by SDHIs will prevent the oxidation of succinate to fumarate in the Tricarboxylic Acid Cycle - TCA, leading to succinate accumulation. (c) Inhibition of αKG-dependent dioxygenases, the histone demethylases (HDM), prolyl hydroxylase (PHD) and the eleven translocation (TET) 5-methylcytosine (5mC) hydroxylases that are directly involved in tumorigenesis, leading to histone alterations and DNA methylation. Adapted: Dalla Pozza *et al.* (2019); Sierotzki; Scalliet (2013); Xiao *et al.* (2012); Zhu *et al.* (2014).

The flutolanil SDHIs classified in ANVISA category 5 (Min. da Saúde 2022a), increased mitochondrial membrane potential on exposure to SDHIs in kidney cells. This infers the difficulty of measuring the possible impacts and risks of certain SDHIs on human health, mainly because they are chemical products with different levels of toxicity, requiring more effective prevention and control measures (Van Der Stel *et al.* 2020).

Exposure to SDHIs and epigenetic mechanisms

In this sense, we represent in schematic how exposure to SDHIs can block the human SDH enzyme (*Figure 2a*), causing the accumulation of succinate (*Figure 2b*) and inducing epigenetic changes that regulate gene expression, leading to the development of diseases (*Figure 2c*). The selected articles demonstrated that the concentration of metabolites produced by the enzymatic reactions involved in the TCA cycle, primarily succinate accumulation, controls the epigenetic regulation of SDH by inhibiting several alpha-ketoglutarate (KG)-dependent dioxygenases involved in histone acetylation and methylation and DNA methylation. In this way, we show how SDHIs fungicides can cause epigenetic changes by inhibiting the human SDH enzyme (Xiao *et al.* 2012; Tretter *et al.* 2016; Sajnani *et al.* 2017; Bernardo-Castiñeira *et al.* 2019; Moog *et al.* 2020; Zhao *et al.* 2020).

Diseases associated with epigenetic regulation of SDH

The selected studies revealed that DNA methylation, histone and DNA demethylation are the primary epigenetic mechanisms that regulate SDH gene expression in cancer, gastrointestinal, paraganglioma, and neuroendocrine tumors. (*Table 2*).

DISCUSSION

The general structure of SDH

Located on the inner membrane of mitochondria, the SDH holoenzyme is formed by four subunits, SDHA, SDHB, SDHC and SDHD, and two assembly factors, SDHF1 and SDHF2 (Moosavi *et al.* 2020). The SDHA subunit catalyzes succinate to fumarate in the TCA Cycle, SDHB is involved in the oxidation of ubiquinone to ubiquinol in ETC, while SDHC and SDHD are primarily responsible for anchoring the SDH pro-

Table 2. Studies related to epigenetic regulation of SDH associated with cancer and other diseases.

Epigenetic modification - Disease	Study *In vivo* or *in vitro* Tissue/animal	Results	Reference
DNA methylation - Cancer	Review	In cancer cells, the accumulation of succinate causes competitive inhibition of several alpha-ketoglutarate (αKG)-dependent dioxygenase	Dalla Pozza et al. (2019)
Histone acetylation - Cancer	*In vitro* and *in vivo* Cell cultures Te human P493-6 B cell line (a gif from C. V. Dang)	Myc triggers a regulatory cascade in cancer cells that leads to H3K4me3 activation and gene expression. Acetylated SDHA at Lys 335 contributes to tumour growth	Li et al. (2020)
DNA and Histone Demethylation / DNA Methylation - Cancer	Review	'Oncometabolites' including the tricarboxylic acid cycle metabolites succinate and fumarate may promote tumorigenesis by altering the epigenome	Nowicki et al. (2015)
	Review	Citrate accumulation can give rise to epigenetic modifications that can promote cancer development and progression	Sajnani et al. (2017)
	In vivo Injection, siRNAs targeting mouse	Accumulation fumarate and succinate, leading to inhibition of multiple a-KG-dependent dioxygenases and consequent alterations of genome-wide histone and DNA methylation	Xiao et al. (2012)
DNA methylation - Tumors gastrointestinal	Tumour tissue and adjacent normal tissue (31/32 cases) and blood when available (21/32 cases)	SDHC promoter methylation was identifed in 6 (18.7%) tumours. All 6 SDHC epimutation cases presented with SDH defcient wild type gastrointestinal stromal tumour and 3/6 cases had multiple primary tumours	Casey et al. (2019)
	Archival formalin-fixed paraffin-embedded (FFPE) tumor and reference tissues	Divergence between the DNA methylation profiles of SDH-deficient GIST (n = 24) versus KIT tyrosine kinase pathway–mutated GIST (n = 39)	Killian et al. (2013)
	Formalin-fixed, paraffin-embedded (FFPE) GISTs tissue	SDH gene mutations and promoter methylation may contribute to the loss of SDH protein expression in sporadic SDH-deficient GISTs	Shi et al. (2019)
	A 25-year-old woman had multifocal, epithelioid gastric GIST	An additional case of dSDH GIST without SDHx mutation but harboring hypermethylation in the SDHC promoter, thus confirming the complexity of the molecular background of this subtype of gastrointestinal stromal tumors	Urbini et al. (2015)
DNA and Histone Demethylation - Tumors gastrointestinal	Review	Recent advances in the understanding of how metabolic enzyme mutations and oncometabolites drive human cancer with an emphasis on SDH mutations and succinate in WT GISTs	Zhao et al. (2020)
DNA methylation - Neuroendocrine Paraganglioma	Tumor and blood samples were obtained from patients with paragangliomas and pheochromocytomas	High levels of PCDHGC3 promoter methylation were validated in primary metastatic SDHB-PPGLs, it was found amplified in the corresponding metastases, and it was significantly correlated with PCDHGC3 reduced expression	Bernardo-Castineira et al. (2019)
Histone Demethylation and DNA methylation - Neuroendocrine Paraganglioma	Formalin-fixed paraffin-embedded (FFPE) tissue samples of head and neck paragangliomas (PGL), pheochromocytomas (PCC), leiomyomas (LM) and leiomyosarcomas (LMS)	Increased methylation of H3K9me3 occurred predominantly in the chief cell component of SDH mutant tumors, while no changes were seen in H3K4me3 and H3K27me3	Hoekstra et al. (2015)
Histone Demethylation and DNA methylation - Neuroendocrine Paraganglioma	Review	Jumonji C domain-containing histone lysine demethylases (KDM2-7) are epigenetic regulators of chromatin	Tretter et al. (2016)
Histone Demethylation - Neuroendocrine Paraganglioma	Review	Several epigenetic regulators are mutated in PPGL tumors, including ATRX which 277 appears to be associated with aggressive disease	Bjorklund et al. (2018)
Histone deacetylation and DNA methylation - Neuroendocrine Paraganglioma	Review	The description of the hypermethylated phenotype of SDH-deficient tumors revealed the possibility of innovative epigenetic therapies involving DNA or histone demethylating agents	Moog et al. (2020)

tein in the inner portion of the mitochondrial membrane (Zhao *et al.* 2020).

The TCA cycle generates ATP by glucose oxidation, and metabolites for numerous anabolic pathways. SDH catalyzes one of the eight steps in the TCA cycle (Sierotzki and Scalliet 2013). SDHA oxidizes succinate at the FAD binding site, forming FADH2 and leaving free fumarate to exit the protein. The electrons from FADH2 are transferred to the Fe – S clusters of SDHB, to the quinone binding site (Qp) in SDHC and SDHD in the inner membrane, resulting in the total reduction of ubiquinone to ubiquinol (Tretter *et al.* 2016; Moosavi *et al.* 2020).

SDHIs in agriculture: Brief history and mode of action

Currently, 22 SDHI compounds are listed in the Brazilian Committee for Action on Fungicide Resistance (FRAC-BR): 1. Phenyl-benzamide (Benodanil, Flutolanil and Mepronil); 2. Phenyl-oxo-ethyl thiophene amide (Isofetamide); 3. Furan-carboxamide (Fenfuram); 4. N-cyclopropyl-N-benzyl-pyrazole-carboxamide (Isoflucipram); 5. N-methoxy-(phenyl-ethyl)-pyrazole-carboxamide (Pidiflumethofen); 6. Oxatin-carboxamide (Carboxin and Oxycarboxin); 7. Pyrazine-carboxamide (Piraziflumide); 8. Pyrazole-4-carboxamide (Benzovindiflupyr, Bixafen, Fluindapyr, Furametpyr, Impirfluxam, Isopyrazam, Penflufem, Penthiopyrad and Sedaxane); 9. Pyridine-carboxamide (Boscalide); 10. Pyridinyl-ethyl benzamide (Fluopyram) and; 11. Thiazole-carboxamide (Tifluzamide) (FRAC 2022).

SDHI fungicides have a different structure, but they share an essential common feature, which is the amide bond used for classification. Furthermore, they can be classified into two main categories: (1) those that bind succinate (e.g. malonate) and, (2) those aimed at crop protection, bind ubiquinone (e.g. carboxamides) (Sierotzki and Scalliet 2013).

The diversity of SDHI fungicides and the biological spectrum exhibited by them is due to the high degree of variation in SDHC and SDHD between species (Sierotzki and Scalliet 2013). The physicochemical properties of SDHIs allow them to be used in a wide range of applications, including seed treatment, foliar and soil irrigation, for the control of fungi in a variety of crops, e.g. potato, grape, soybean, rice, wheat, corn and others (Tian *et al.* 2020).

Susceptibility to diseases influenced by the toxic effect of SDHIs

The results obtained with the research at AGROFIT for the chemical groups carboxamide and benzamide, which make up the SDHIs fungicides, showed that there are several products registered by MAPA and that they have been used in different MAPA 2022. Fungicides are routinely applied as preventive crop protection, in the various stages of plant growth and in post-harvest storage (Tian *et al.* 2020).

Among the various fungicides used in agriculture, SDHIs stand out due to their broad spectrum of action (Sierotzki and Scalliet 2013; Gulkowska *et al.* 2014). This group of fungicides acts by inhibiting the SDH enzyme of plant pathogens, with SDH being an essential and evolutionarily conserved component of the mitochondrial respiratory chain of living organisms (Brenet 2021).

In this context, some concerns have been raised about the agricultural importance of SDH in the development of fungicides, which may have the potential to inhibit this enzyme not only in plant pathogens, but also in non-target organisms (Van Vranken *et al.* 2015; Bénit *et al.* 2019; Brenet *et al.* 2022). The SDH enzyme from humans, bees, earthworms, and fungi was sensitive to the eight SDHIs tested, indicating that the SDHB-D subunits that comprise the ubiquinone reduction site are highly conserved (Bénit *et al.* 2019).

According to ANVISA, the degree of toxicity of these substances varies from Category 2 - Highly toxic product, which can be fatal if ingested, in contact with the skin and/or inhaled, to Category 5 - Product unlikely to cause acute harm (Min. da Saúde 2022a). Is extremely dangerous to compare the toxic effect of SDHIs *in vitro* with the concentrations of SDHIs applied in agricultural cultivation. According to these authors, the concentration of the fungicide at the spray nozzle outlet and the mist cloud per hectare can be reliably determined. However, the final exposure is determined by a number of uncontrollable factors such as propagation conditions, soil type, vegetation cover, and so on (Bénit *et al.* 2019).

The neurotoxicity of bixafen, one of the most recently released SDHI fungicides, was assessed, the results found by these researchers through *in vivo* analysis showed that the central nervous system is highly sensitive to bixafen. According to them, this SDHI is neurotoxic in vertebrates and causes defects in neurological development, which can cause microcephaly and impair the growth of motor neuron axons (Brenet 2021). Therefore, strategies to help protect against the neurotoxicity of these substances must be adopted to ensure the protection of human health.

Mutations of SDH-encoding genes lead to blockade to varying degrees of SDH activity, being associated with cancer (Zhao *et al.* 2020) and a wide spectrum of diseases (Hoekstra *et al.* 2015). However, that patients without mutations in all four SDH subunits may also have diseases caused by the loss of SDH enzyme activity (Van Vranken *et al.* 2015). In patients with neurological or neurodegenerative diseases caused by ETC impairment and/or hypersensitivity to oxidative insults, such as Friedreich's ataxia (FRDA) and familial Alzheimer's disease, a pre-existing mitochondrial defect, such as partial SDH dysfunction, increases susceptibility to SDHIs (FAD) (Bénit *et al.* 2019).

SDH inhibition may occur as a compensatory mechanism due to the presence of pyruvate, which was sufficient to supply the TCA cycle to the succinate oxidation step, limiting NADH depletion. Although not statistically significant, flutolanil and mepronil appear to slightly increase mitochondrial membrane potential in kidney cells, while SDH compounds increased cellular oxygen consumption, resulting in internal mitochondrial hyperpolarization (Van Der Stel *et al.* 2020).

Results of the characterization analysis of metabolic changes resulting from *in vivo* exposure to the SDHIs boscalide and fluxapyroxad in rats, indicated that the SDH activity inhibiting action by both compounds did not result in changes in succinate or lactate levels. As a result, these authors proposed the existence of multiple biochemical pathways capable of replacing SDH's decreased activity and maintaining homeostasis (Kamp *et al.* 2021).

Inhibiting SDH can limit the ability of the grid to lose carbon in the form of CO_2 emissions, forcing it to operate with greater carbon efficiency. The simulation of partial SDH inhibition produced similar but more moderate effects than complete SDH inhibition, in-

dicating an effect dependence and partial or, in some cases, complete restoration of the flow ranges. Thus, the results found by (Bénit *et al.* 2019; Van Der Stel *et al.* 2020; Zhao *et al.* 2020; Brenet *et al.* 2021; Kamp *et al.* 2021) on susceptibility to diseases influenced by the toxic effect of SDHIs, highlight the risks of exposure to these fungicides that can contribute to the acceleration of disease progression, especially in people who already have a partial deficiency in SDH.

Diseases associated with epigenetic regulation of SDH

Comparing the results presented in the present work (Table 2), we conclude that oncometabolites are involved in the emergence and development of various tumors and human diseases that involve epigenetic alterations (DNA and histone methylation demethylation, and histone acetylation). Concerning the negative points, it is observed that more research is required to deepen knowledge in this area, primarily aiming at innovative therapeutic strategies for the treatment of cancer and other diseases through clinical trials or in isolated individuals.

Gene expression can be modulated epigenetically through methylation of cytosine residues in DNA and chemical modification of histone tails (Björklund and Backman 2018). These processes are essential for normal health, activating and suppressing genes that are vital for cellular functions (Nowicki and Gottliev 2015). Changes in DNA methylation patterns, such as hypermethylation of CpG islands, have been observed in human cancers, this process being modulated by DNA methyltransferases (DNMTs) that add methyl groups (Nowicki and Gottliev, 2015), and Ten-Eleven translocation (TET) - protein from the dioxygenase family that removes methyl groups, converting methyl cytosine (5mC) to 5-hydroxymethylcytosine (5hmC), then 5hmC to 5-formylcytosine (5fC) and finally 5fC to 5-carboxylcytosine (Nowicki and Gottlieb 2015; Dalla Pozza *et al.* 2020).

SDH-deficient gastrointestinal stromal tumors (GISTs) exhibit specific clinicopathological features, consist of epithelioid tumor cells, often metastasize to lymph nodes and liver, occur among children and adults, with a female predominance (Shi *et al.* 2019). The methodology proposed by Casey *et al.* (2019) for the diagnostic test of SDHC epimutation in GISTs, allowed to identify 4 cases of SDHC tumor promoter hypermethylation (Casey *et al.* 2019).

The case study by Urbini *et al.* (2015) revealed that epigenetic regulation by DNA methylation of CpG islands of the SDHC promoter was observed as an alternative mechanism underlying the lack of SDH complex in GIST, he loss or inactivation of SDHB expression in sporadic succinate dehydrogenase deficient patients with GISTs is caused by promoter hypermethylation that can cause gene silencing of the tumor suppressor gene (TSGs) and lead to the formation and development of tumors (Shi *et al.* 2019).

In the PPGL neuroendocrine tumor containing SDH mutations, a hypermethylator phenotype is associated with downregulation of key genes involved in neuroendocrine differentiation (Aldera and Govender 2018). The DNA methylation profile revealed a hypermethylation phenotype in SDHx - PPGLs and revealed that succinate acts as an oncometabolite, inhibiting 2-oxoglutarate-dependent dioxygenases such as histone and DNA demethylases, causing epigenetic changes in several SDHx - paraganglioma traits (Bernardo-Castiñeira *et al.* 2019).

The epigenetic mechanisms that control cell proliferation and differentiation are regulated by oncometabolites, resulting from the enzymatic reactions involved in the energy metabolism of the TCA cycle (Sajnani *et al.* 2017). Changes in the abundance of metabolites such as acetyl-CoA and S-adenosyl methionine (SAM), which are substrates for key biochemical reactions such as acetylation and methylation, can affect the epigenetic status of the entire genome (Bernardo-Castiñeira *et al.* 2019; Zhao *et al.* 2020).

Succinate accumulation inhibits PHDs, resulting in the stabilization of hypoxia-inducible factor-1α (HIF1α) proteins. Inhibiting PHD allows HIF subunits to escape degradation and bind to HIF to form a heterodimer, which forms an active complex under hypoxic conditions and acts on HIFβ target genes that regulate biological processes such as cell survival, angiogenesis, cell growth, proliferation, and glycolysis (Sajnani *et al.* 2017; Moog *et al.* 2020).

In addition to the currently widely used SDHI fungicides, new SDHIs with greater action potential are being tested and developed. In this regard, in terms of health care and the environment, it is recommended to avoid the use of chemical fungicides as much as possible, instead opting for alternative methods of controlling plant diseases, such as biological control and crop rotation, when possible.

CONCLUSIONS

In view of the studies presented in this review, we understand that further *in vitro* and *in vivo* to identify the risks of developing cancer, gastrointestinal and neuroendocrine tumors - pheochromocytoma / paraganglioma associated with SDHI exposure, analyses should be performed to assess susceptibility to diseases influenced by the toxic effect of SDHIs.

Based on our interpretation of the articles cited in

this review, we discovered that MAPA has recommended many SDHI fungicides for various agricultural crops, which can significantly benefit production by controlling plant diseases. Despite their agricultural benefits, SDHIs have been shown in studies to inhibit the SDH enzyme in humans and many other living things.

According to the research presented in this review, the human SDH enzyme is sensitive to some of the SDHIs tested, and the central nervous system is highly sensitive to bixafen, which can cause microcephaly and neurodevelopmental defects. Furthermore, other research has linked epigenetic regulation of the SDH enzyme to the development of cancer, gastrointestinal tumors, and neuroendocrine - pheochromocytoma/paraganglioma.

As a result, additional research should be conducted to assess the action potential of SDHIs on the human SDH enzyme, as well as their health and environmental risks. In this regard, the findings of this review highlighted some recent concerns about the safety of these agrochemicals, which will help with future research to ensure protection against the toxic effects of SDHIs.

CONFLICTS OF INTEREST

Declaration of competing interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this.

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