INSERM/CNRS
UMR 1141S
Paris

Hôpital
Robert Debré
Paris

5 years:

still there!

Mitochondrial diseases:
A number of puzzling questions

SDH deficiency:
A paradigm of these questions

Pesticides target biodiversity with human health as a side effect

SDH… SDHI
A madness denounced as early as 1950

Pesticides:
Failure of toxicological science prediction

Mitochondriotoxic pesticides:
A deadly cocktail

Paule Bénit

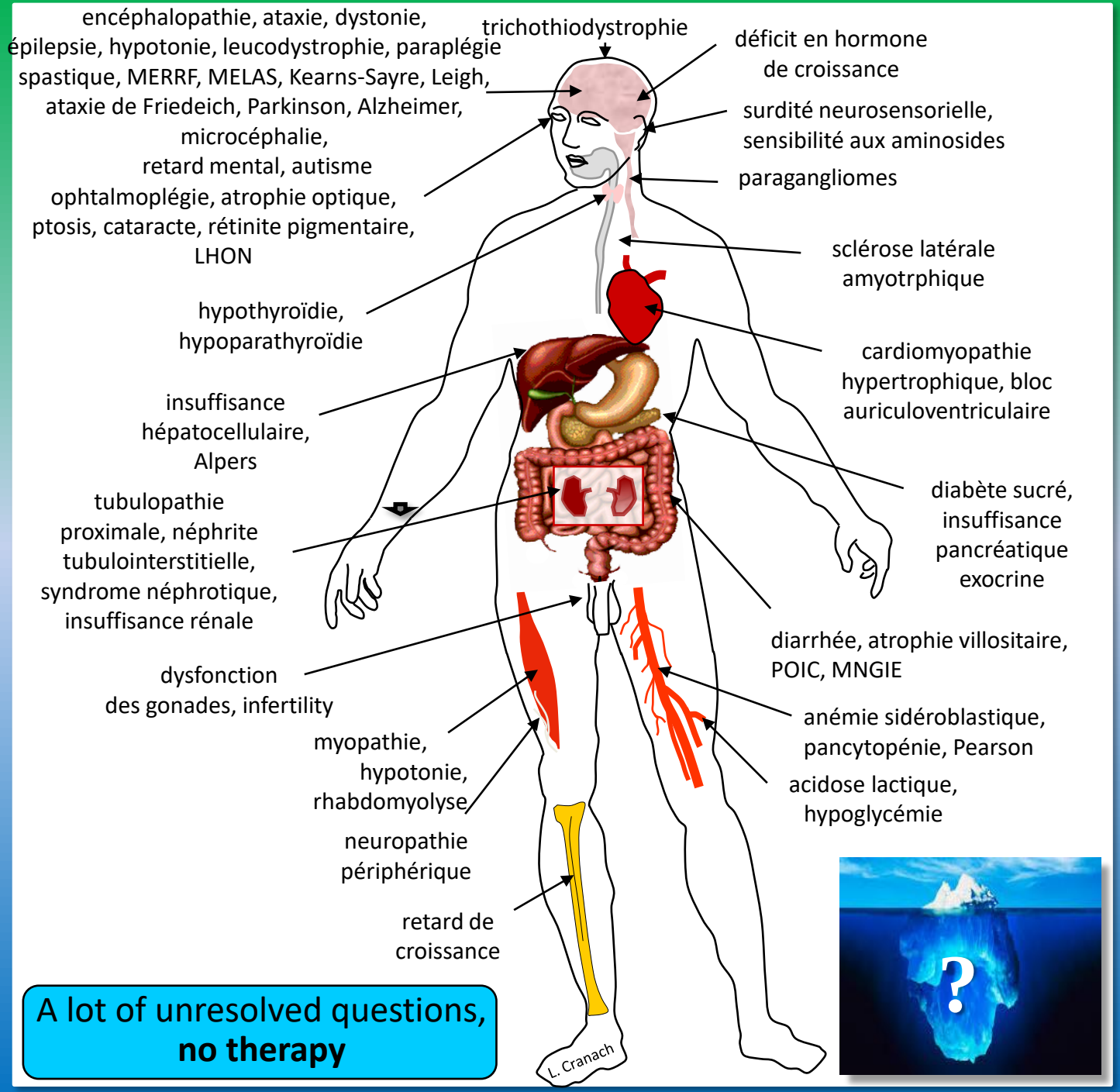
Pierre Rustin

35 years of mitochondrial disease study

- Until 1985, considered impossible

- **2019, an ever-increasing number of diseases**

- Any age
- Any organ (one or more)
- Evolution depending on people



A lot of unresolved questions, **no therapy**

SDH defects : A perfect paradigm for mitochondrial diseases

A First

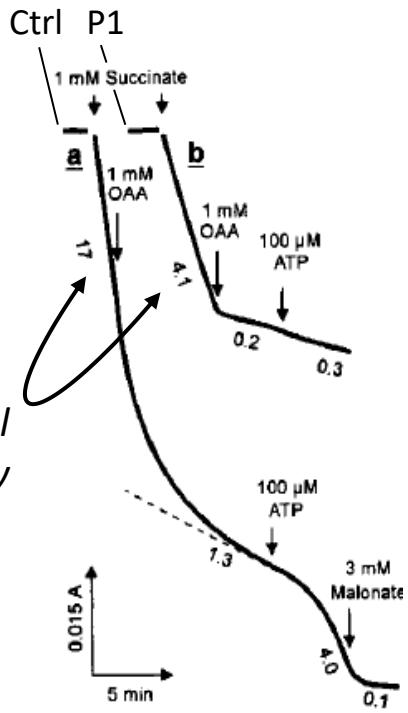
1995

Nature Genetics volume 11 october 1995

Mutation of a nuclear succinate dehydrogenase gene results in mitochondrial respiratory chain deficiency

Thomas Bourgeron, Pierre Rustin, Dominique Chretien, Mark Birch-Machin, Marie Bourgeois, Evani Viegas-Péquignot[1], Arnold Munnich & Agnès Rötig

SDH activity in cultured skin fibroblasts



24% residual SDH activity

*Succinate quinone DCPIP réductase

Béatrice Parfait · Dominique Chretien · Agnès Rötig
Cécile Marsac · Arnold Munnich · Pierre Rustin

2000

Compound heterozygous mutations in the flavoprotein gene of the respiratory chain complex II in a patient with Leigh syndrome

Recessive germline *SDHA* and *SDHB* mutations causing leukodystrophy and isolated mitochondrial complex II deficiency

2012

Charlotte L Alston,[1] James E Davison,[2,3] Francesca Meloni,[4] Francois H van der Westhuizen,[1,5] Langping He,[1] Hue-Tran Hornig-Do,[1] Andrew C Peet,[2,6] Paul Gissen,[7]

Leukoencephalopathy with accumulated succinate is indicative of *SDHAF1* related complex II deficiency

2012

Andreas Ohlenbusch[1*†], Simon Edvardson[2], Johannes Skorpen[3], Alf Bjornstad[4], Ann Saada[5], Orly El
Jutta Gärtner[1] and Knut Brockmann[1*]

Familial neonatal isolated cardiomyopathy caused by a mutation in the flavoprotein subunit of succinate dehydrogenase

European Journal of Human Genetics (2010) 18, 1160–1165
© 2010 Macmillan Publishers Limited All rights reserved 1018-4813/10

Aviva Levitas[1,2,8], Emad Muhammad[1,3,8], Gali Harel[1,3,8], Ann Saada[4], Vered Chalifa Caspi[5], Esther Manor[1,6], John C Beck[7], Val Sheffield[7] and Ruti Parvari*[,1,3,5]

- ***Encephalopathies or severe Cardiomyopathies***
- ***very rares***
- ***Death: <1 à >12 ans***

2000/2001

In adulthood as well…

Mutations in *SDHD*, a Mitochondrial Complex II Gene, in Hereditary Paraganglioma

Bora E. Baysal[1,*], Robert E. Ferrell[2], Joan E. Willett-Brozick[1], Elizabeth C. Lawrence[2], David Myssiorek[5], Anne Bosch[6], Ande…
+ See all authors and affiliations

The R22X Mutation of the *SDHD* Gene in Hereditary Paraganglioma Abolishes the Enzymatic Activity of Complex II in the Mitochondrial Respiratory Chain and Activates the Hypoxia Pathway

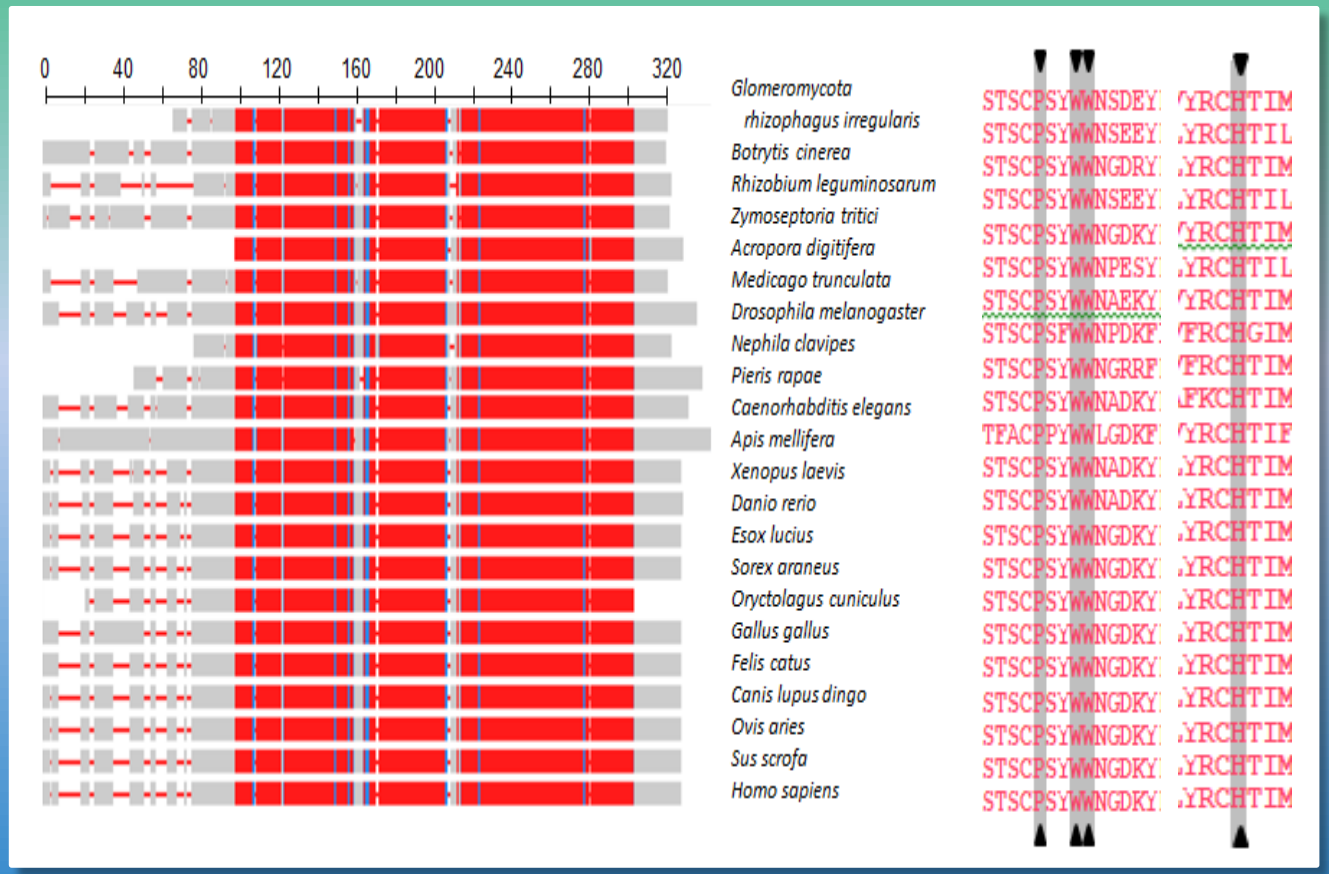
Anne-Paule Gimenez-Roqueplo,[1] Judith Favier,[3] Pierre Rustin,[4] Jean-Jacques Mourad,[5] Pierre-François Plouin,[2] Pierre Corvol,[2,3] Agnès Rötig,[4] and Xavier Jeunemaitre[1,3]

Science, 2000; American Journal Human Genetics 2001

Loss of SDH function (SDHx mutation) can result in:

- Paragangliomas (head and neck, thorax, abdomen and bassin tumours)
- Pheochromocytomas (adrenal medulla)
- Kidney Cancers
- Gastro-Intestinal stromal Tumour (GIST)
- Carney-Stratakis Syndrome

SDH: 4 highly conserved genes through evolution



SDHB

▲ aminoacids involved in SDH**I** binding to SDH

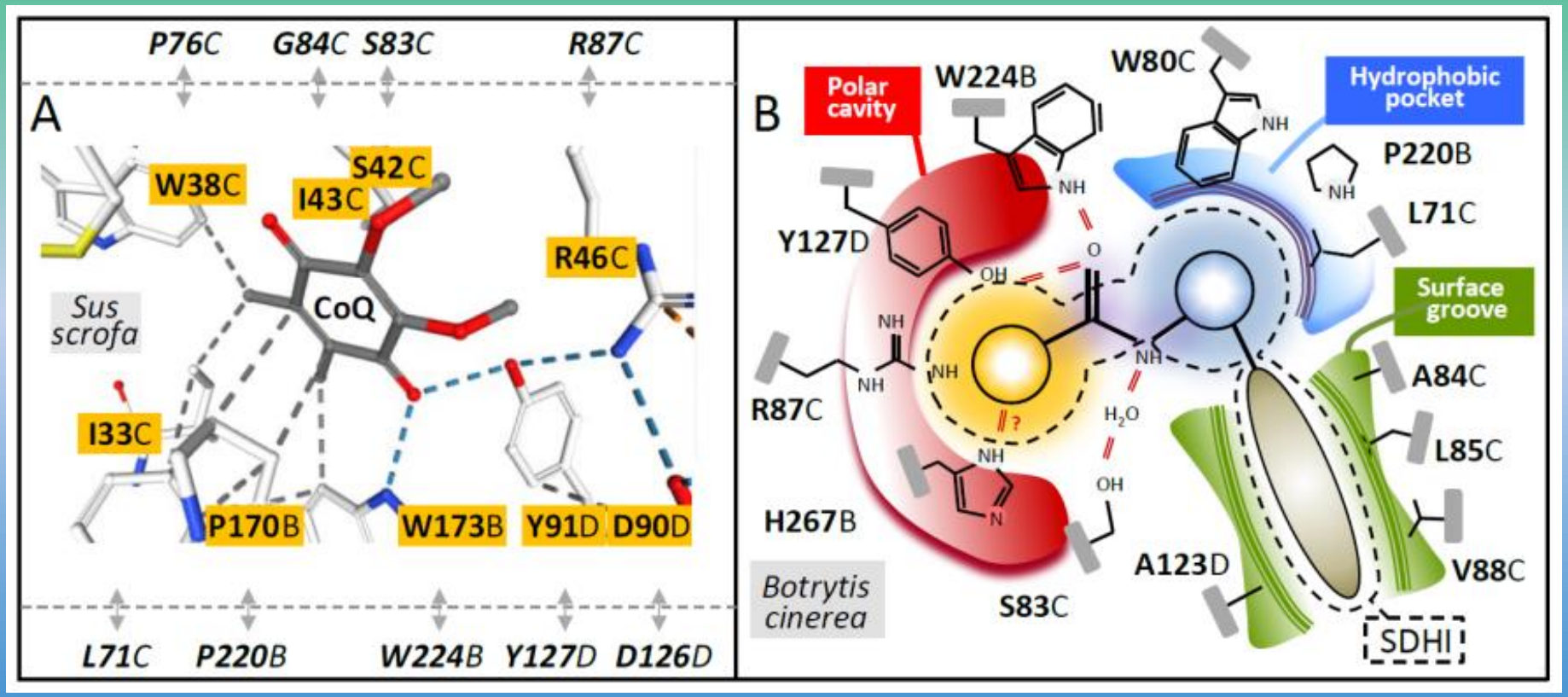
As early as the 50's, it was known that SDH is highly conserved

⇩

SDHI**: cannot have any kind of specificity towards species**

SDHI target SDH

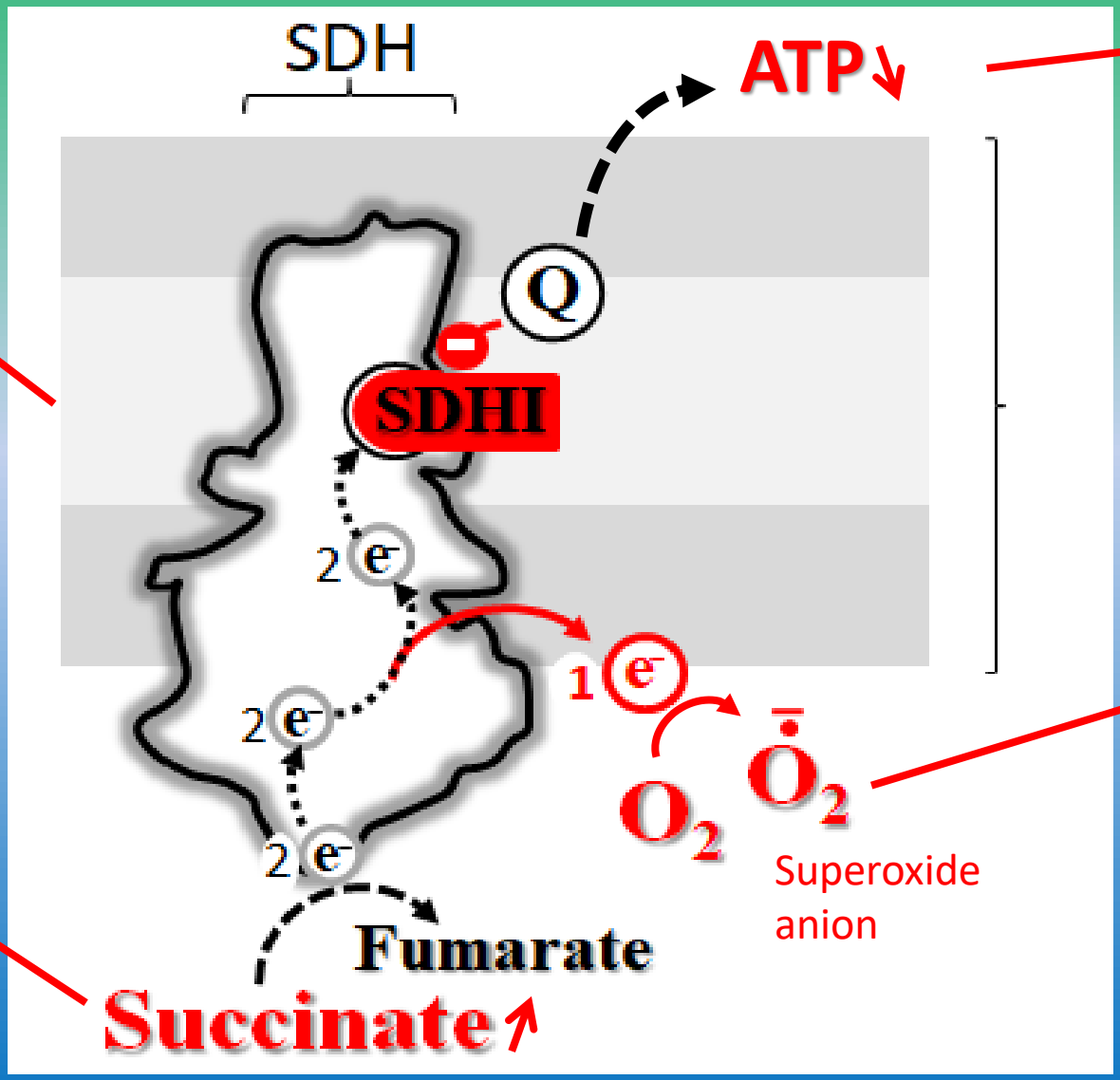
Since the 50's, **no scientific controversy**: SDHI bind the SDH where ubiquinone (CoQ) does



A. Aminoacids of the CoQ binding site involved in SDHI binding *(yellow)*

B. Binding of SDHI (dotted line) on polar (red), apolar (blue) and surface groove (green) of the SDH

POTENTIAL BIOCHEMICAL EFFECTS OF SDHI



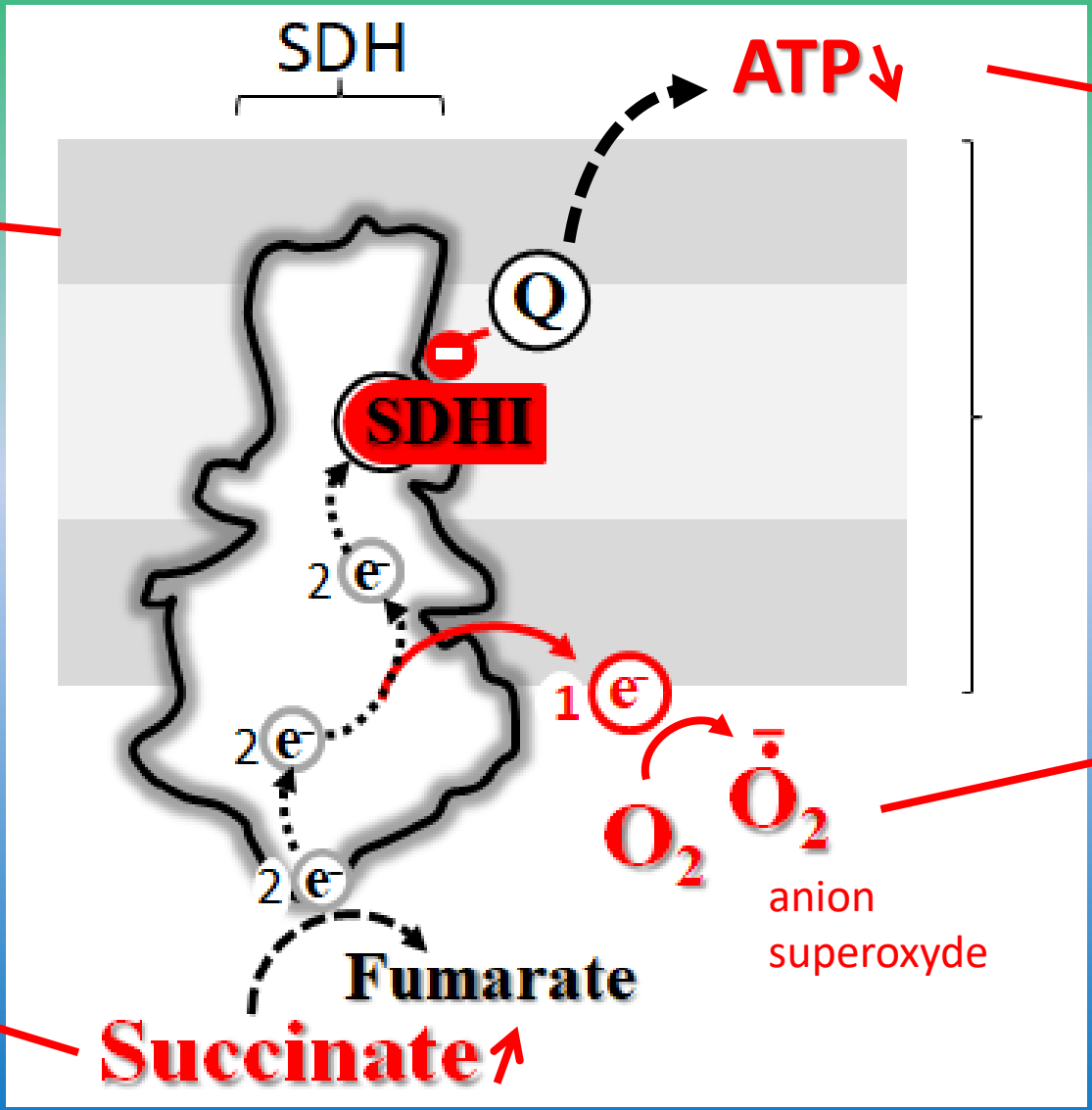
1 Impaired cell respiration

2 Oxidative stress

3 Succinate accumulation

4 Cell lactate excretion

POTENTIAL PATHOLOGICAL CONSEQUENCES OF SDHI



1

According to defect severity, variable neurological or/and muscular impairment, etc

2

Neurological impairment, tumors, cancers

3

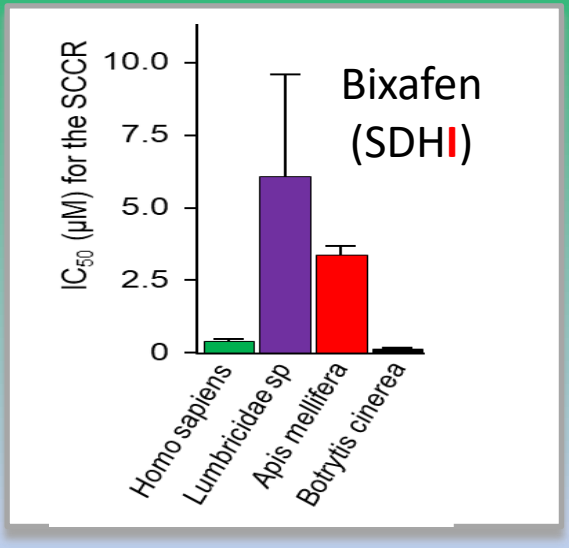
Changes of epigenetics (cancer, developmental anomalies)

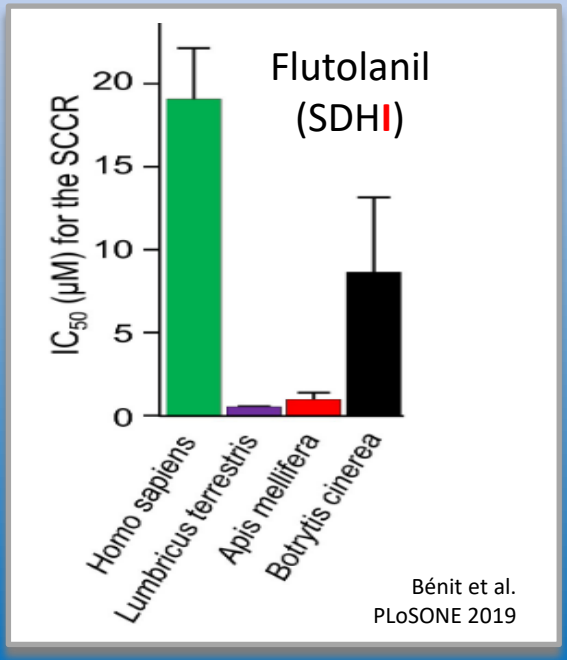
4

Lactic acidosis ↗

Lact/Pyr ratio ↗

Effect of eight SDHI on SDH from four different species

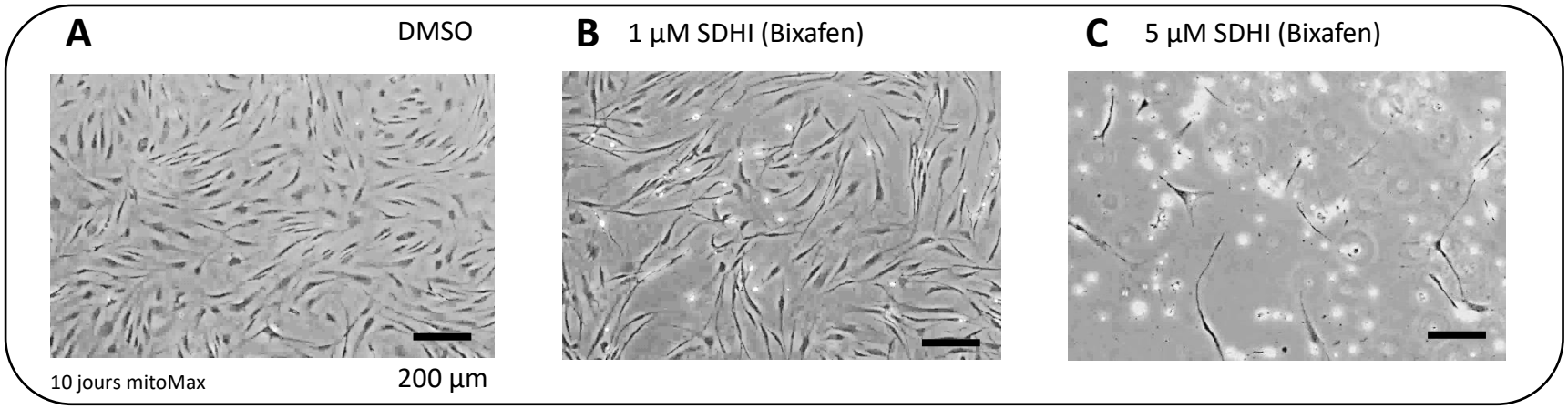




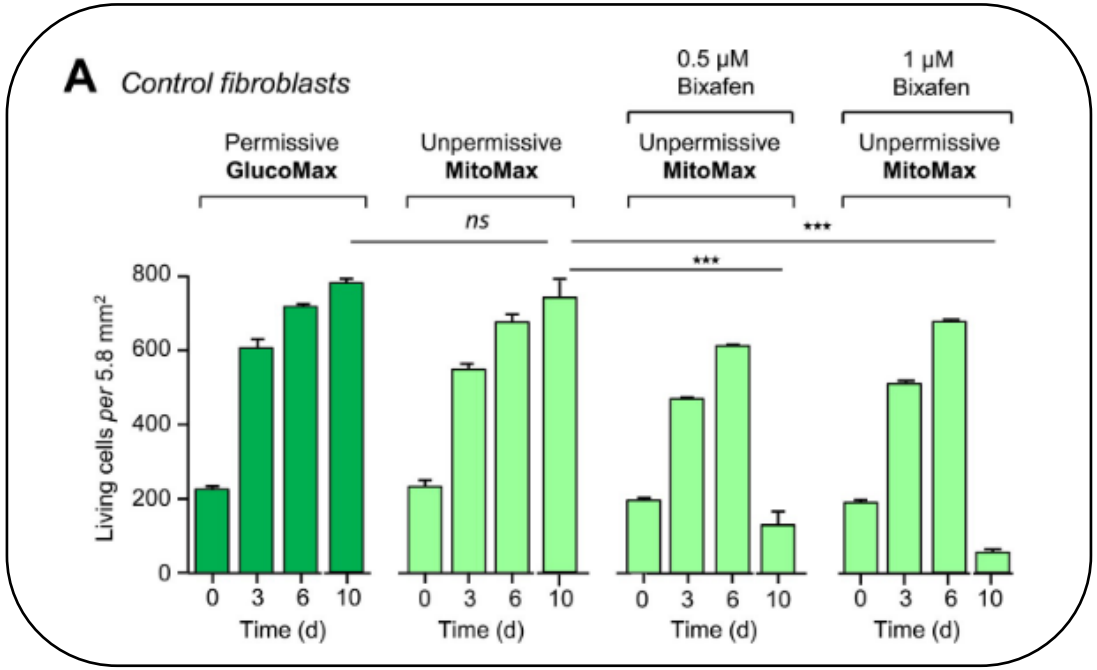
No surprise:
SDHI inhibit SDH regardless of the species

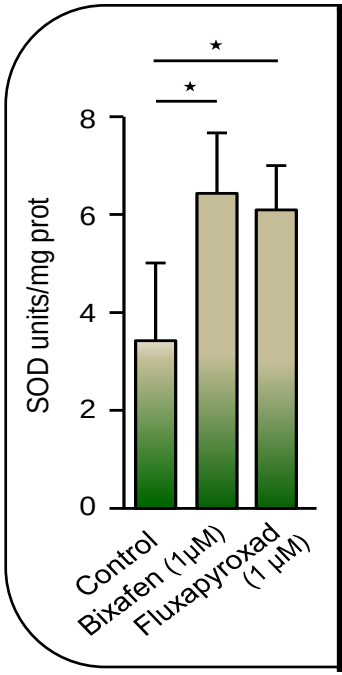
Target specificity?
NO, the latest generation SDHI also inhibit complex III of the respiratory chain

Low concentrations of SDHI readily kill human cells in culture



Control cultured skin fibroblasts





SOD
at day 13.

Cells were cultured in the presence of glucose 1g/l

Bénit et al PloSONE, 2019

Cell death comes much faster if cells are from sick people

SDHA mutant fibroblasts

2 Patients with the very first nuclear gene mutation resulting in a mitochondrial disease: a severe Leucodystrophy presenting as a Leigh Syndrome

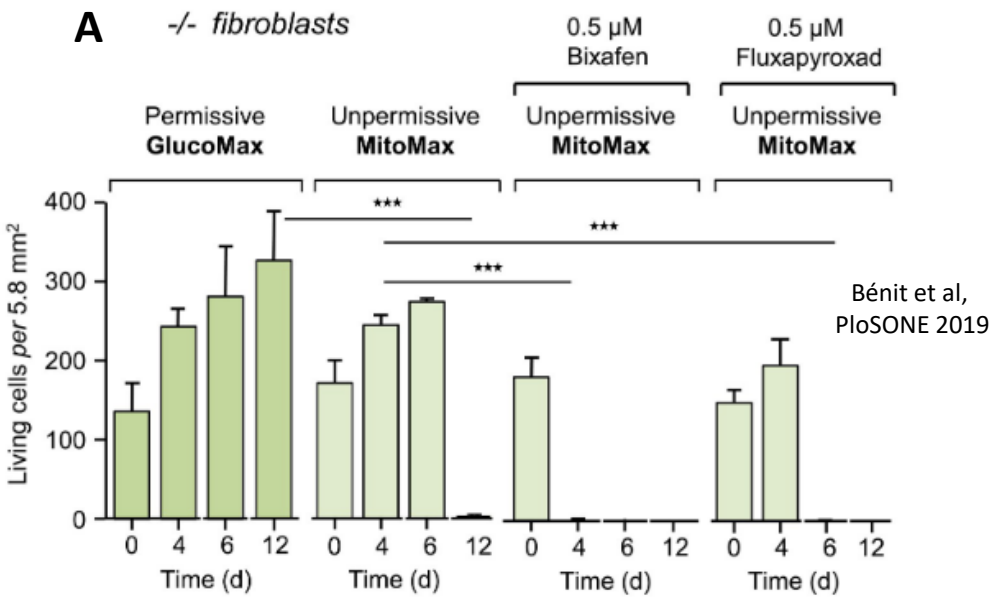
Bourgeron et al. Nat Genet, 1995

Fibroblasts:

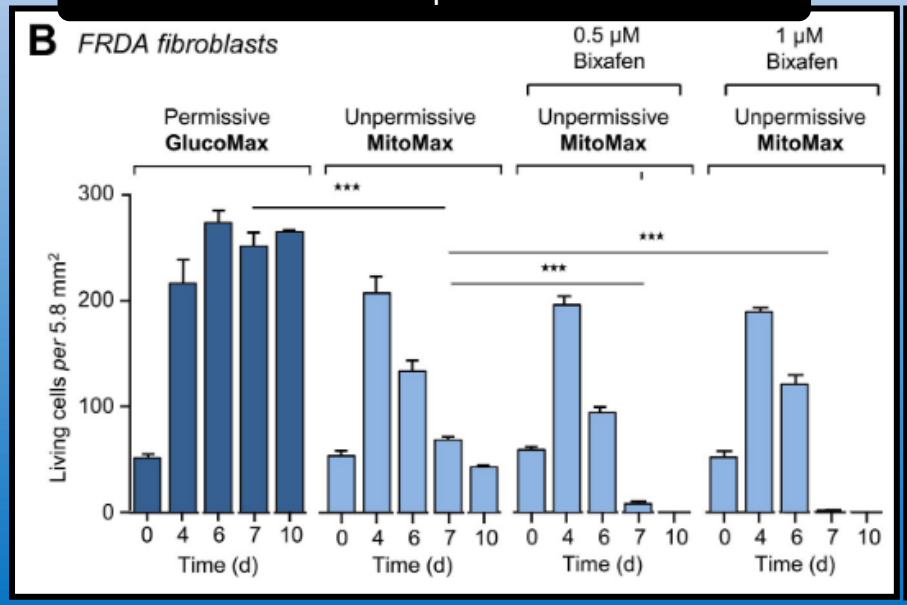
SDH Defect < 20% Briére et al. Hum Mol Genet,2005

Increased total SOD (4 fold)

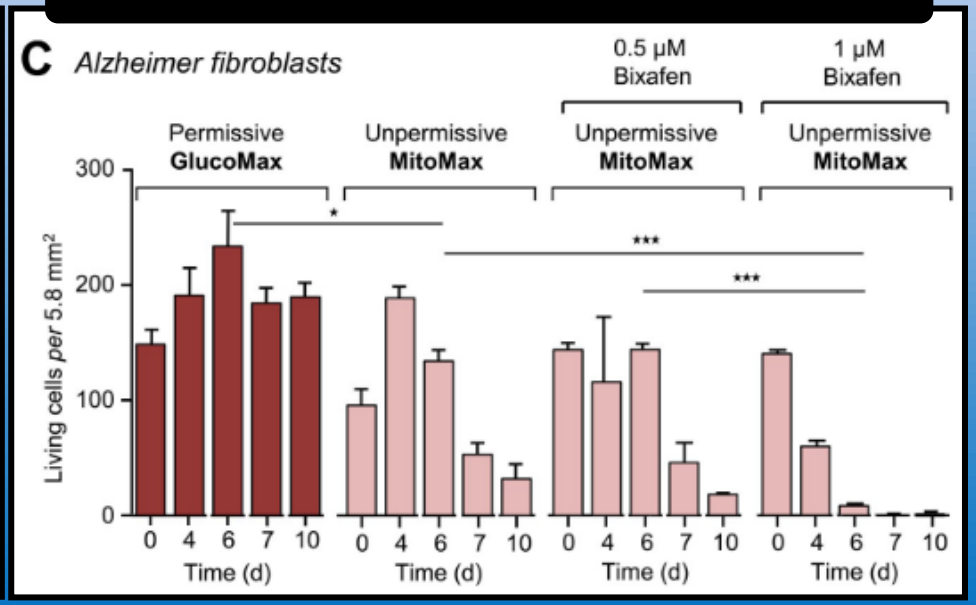
Succinate accumulation (4 fold)



Friedreich ataxia: 1500 patients in France

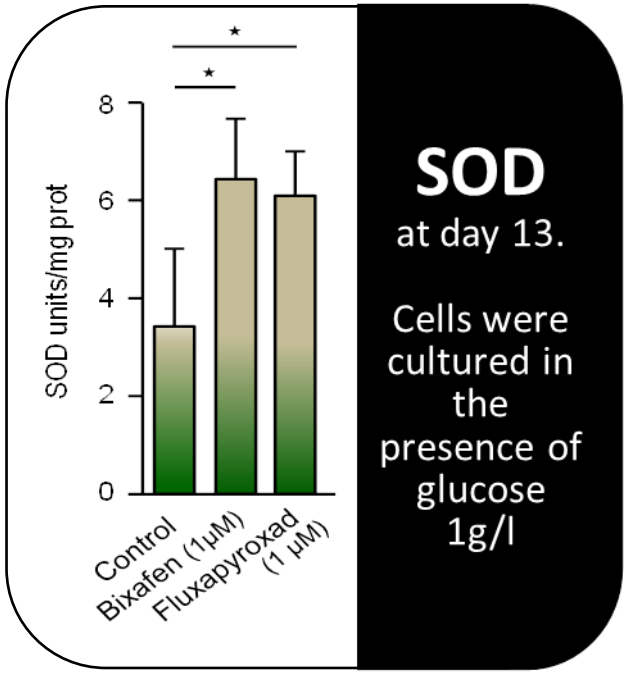


Alzheimer : + than 65 000 individuals less then 65 ans



Oxidative stress is induced by SDHI in human cells but also in a number of different organisms

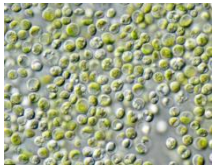
Control cultured skin fibroblasts



Bénit et al, PloSONE 2019

Danio rerio

Chlorella vulgaris



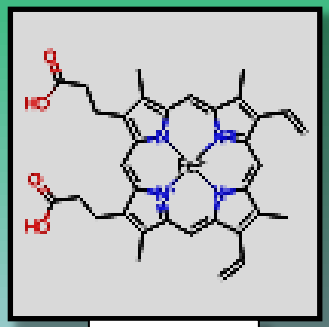
Daphnia magna

SOD, CAT, GSH,GST, lipid pexoxidation

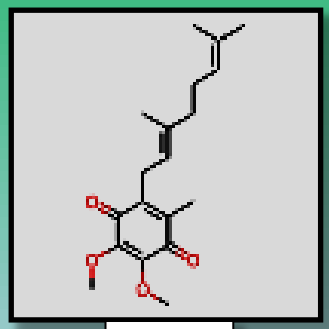
Zang et al, 2017Agricultural Science & Technology
Yao *et al,* 2018 Environ Sci Pollut Res
Yao et al,2018 Chemosphere
Qian *et al,* 2018 Environ Pollut
Qia et al, 2018 Sci Total Environ
Teng et al, 2019,chem Res Toxicol
zhou et al, 2019 Toxicol and environ chemistry
Li et al, 2020 chemosphere
Wang *et al* 2020 Chemosphere
Asakal et al, 2020 Comp Biochem Physiol C Toxicol Pharmacol

The 6 redox carriers of the SDH potential sources of superoxides

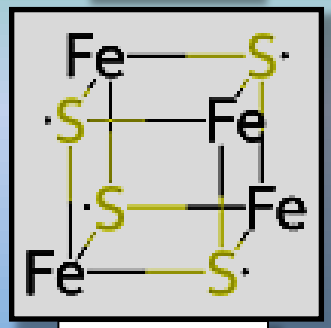
1 FAD⁺, 3FeS, 1 heme *b*-type, UQ



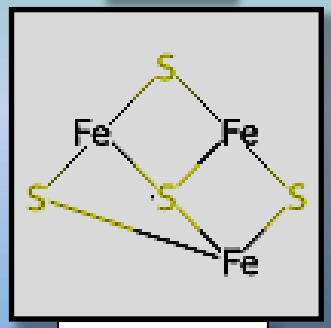
*b*556



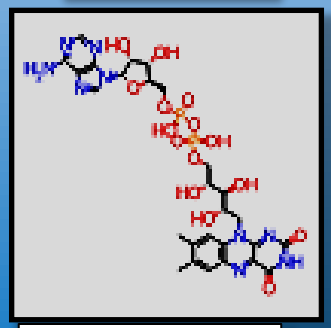
UQ



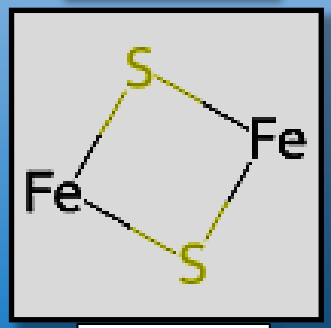
4Fe-4S



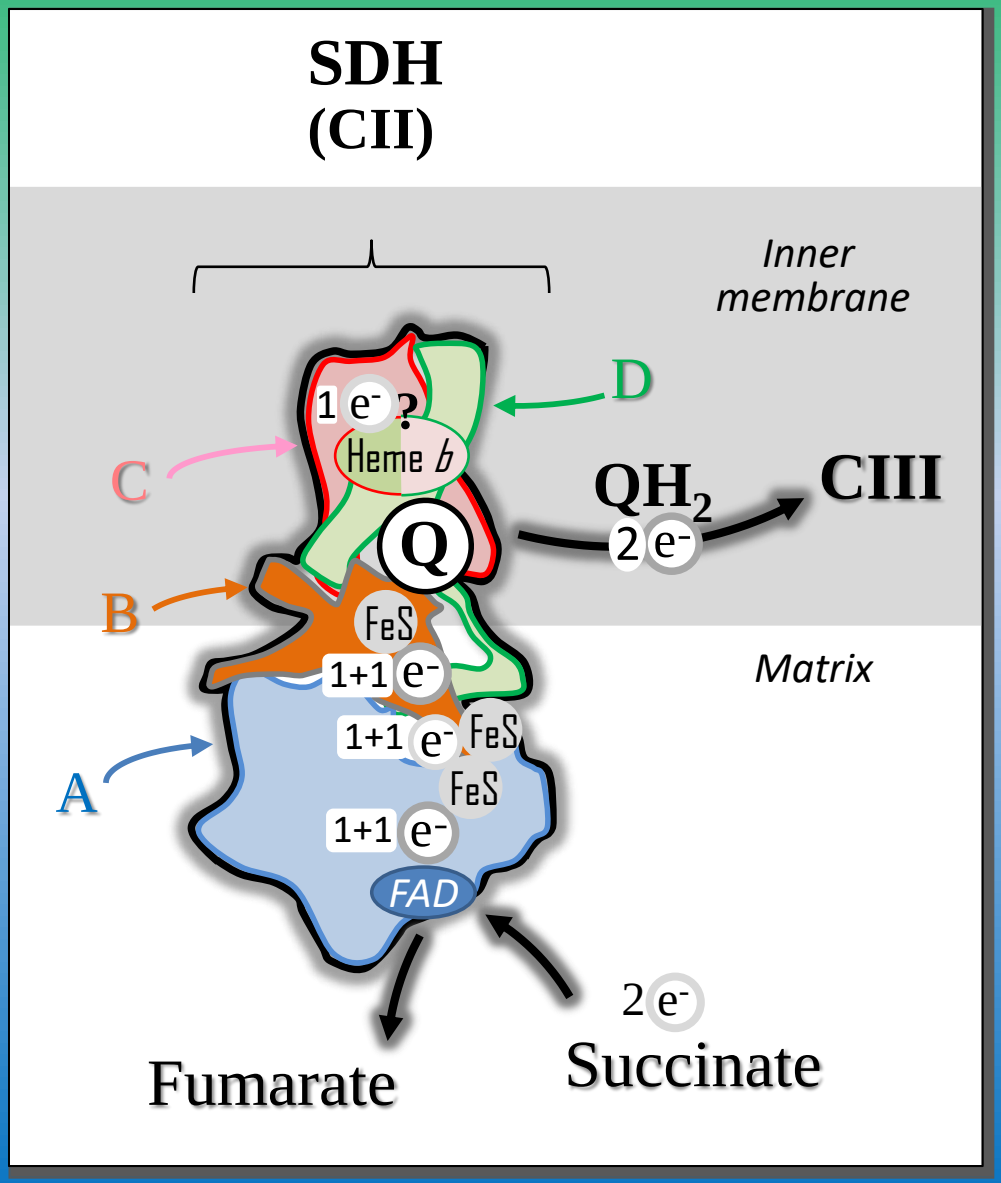
3Fe-4S



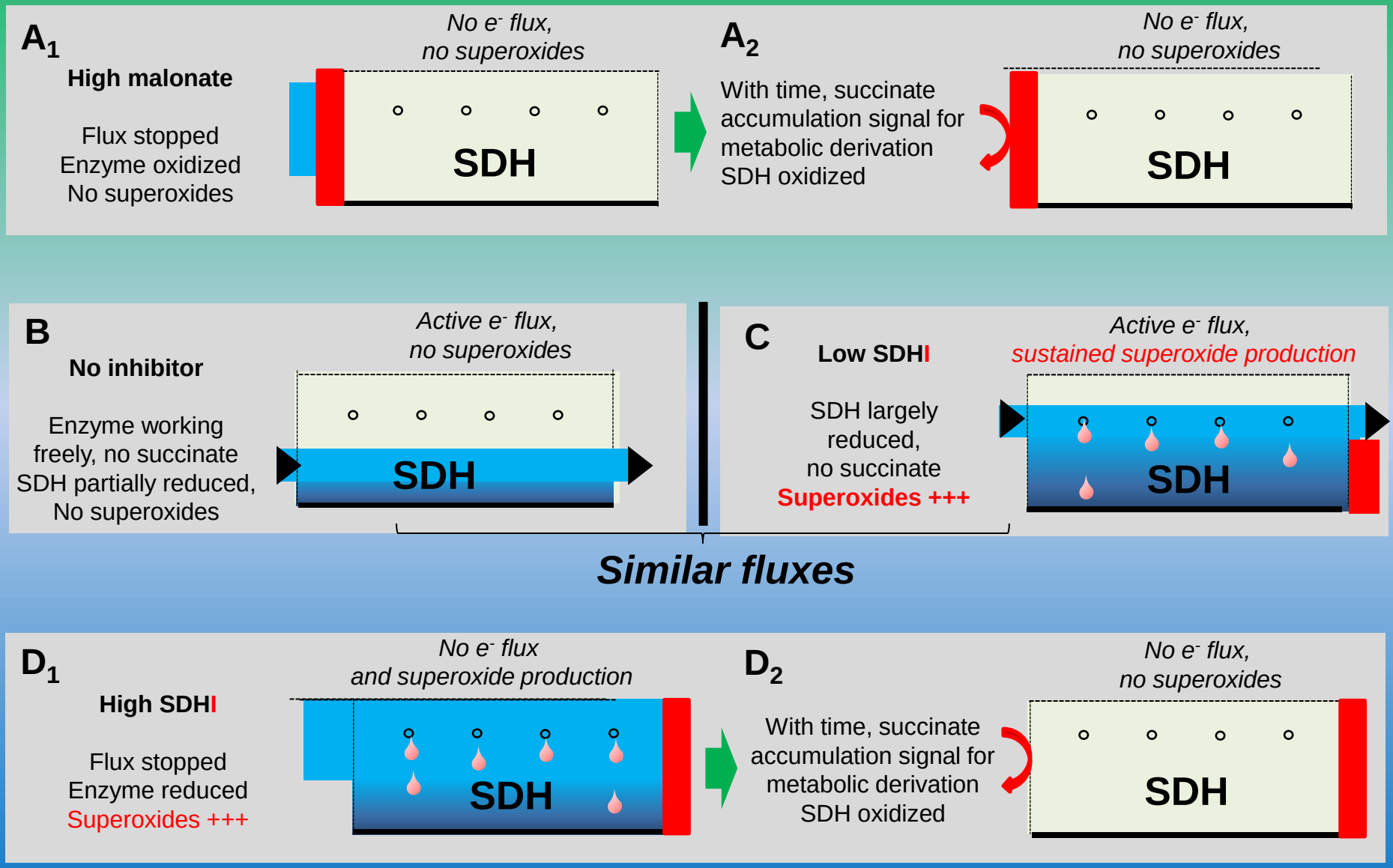
bound-FAD⁺



2Fe2S



Superoxide production depends on SDH redox center reduction



How is that just possible?

Penflufen (SDHI) position paper ECHA

→ *European Chemicals Agency*

REGULATORY TOXICOLOGY

POSITION PAPER

Subject :
Penflufen
Position paper on the proposed classification with H351

Author:

Claire Pecoraro-Mercier, Ph.D.
Regulatory toxicologist

Bayer SAS
CropScience Division
Sophia Antipolis Cedex FRANCE

Date : 20th November, 2017

BUSINESS CONFIDENTIAL

1/21

PROPERTY O

SDHI (penflufen)

(long term dietary administration)

1. Mice: **slightly higher incidence** of adenoma and carcinoma in the **liver** … *when compared to controls*. **Bayer: not treatment-related**

2. Rats: ***higher incidences* of neoplastic lesions were observed** in the **liver**. … specific to the rodent, **Bayer: no relevance to humans**

3. Rats: ***higher incidences* of neoplastic lesions (ovaries, hematopoïetic system, brain)** in some **treated** dose groups… **Bayer: not related to treatment ?!?**

⇨ **Bayer:** Findings **clearly** show that penflufen is **not considered to present a carcinogenic risk** to humans

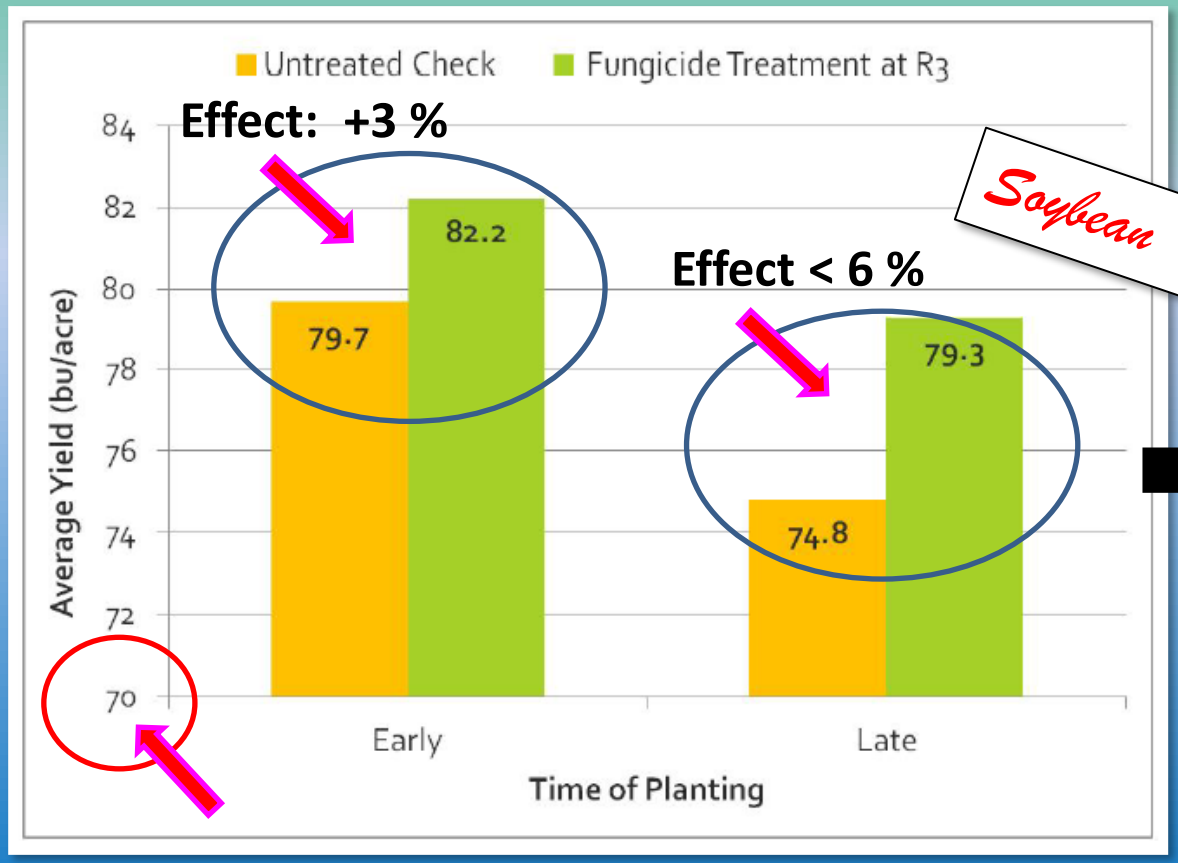
https://monsanto.com/app/uploads/.../fungicide-response-planting-date-soybean.pdf

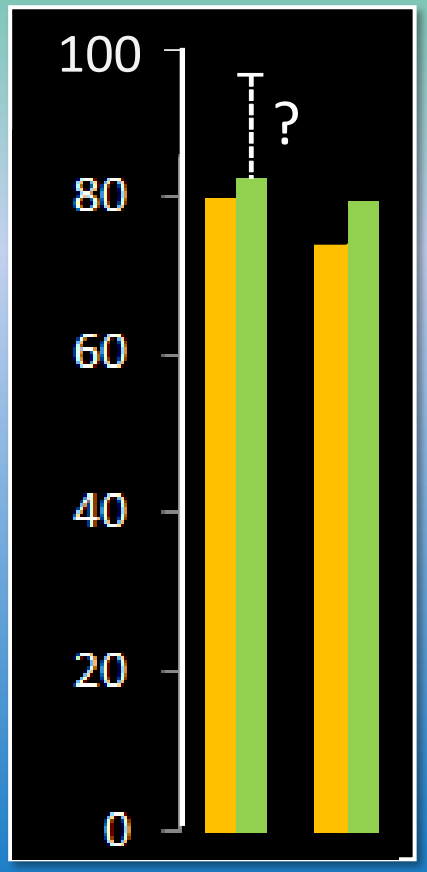
MONSANTO

Demonstration Report

MONSANTO LEARNING CENTER AT MONMOUTH, IL

Foliar fungicide: SDHI plus Strobilurine (inhibition CII & CIII)





Average **soybean yield** based on the time of planting and fungicide application at R3 growth stage in 2016

Fungicide benefits remain below the valuation margins...

Soybean

SCIENTIFIC REPORTS
natureresearch

Received: 31 January 2019
Accepted: 17 July 2019
Published online: 09 September 2019

Neonicotinoid seed treatments of soybean provide negligible benefits to US farmers

Spyridon Mourtzinis[1], Christian H. Krupke[2], Paul D. Esker[3], Adam Varenhorst[4], Nicholas J. Arneson[1], Carl A. Bradley[6], Adam M. Byrne[7], Martin I. Chilvers[7], Loren J. Giesler[5], Ames Herbert[8], Yuba R. Kandel[9], Maciej J. Kazula[10], Catherine Hunt[9], Laura E. Lindsey[11], Sean Malone[8], Daren S. Mueller[9], Seth Naeve[10], Emerson Nafziger[12], Dominic D. Reisig[13], William J. Ross[14], Devon R. Rossman[7], Sally Taylor[8] & Shawn P. Conley[1]

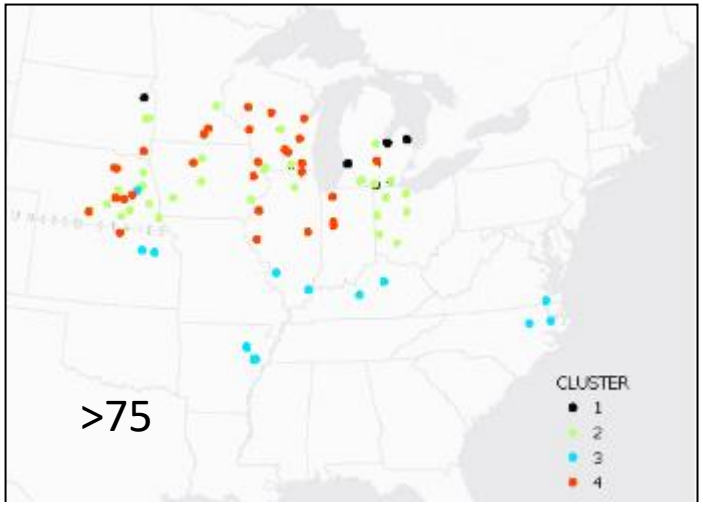
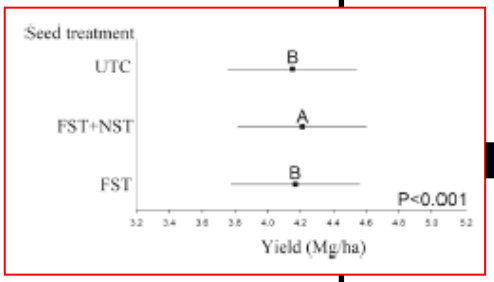
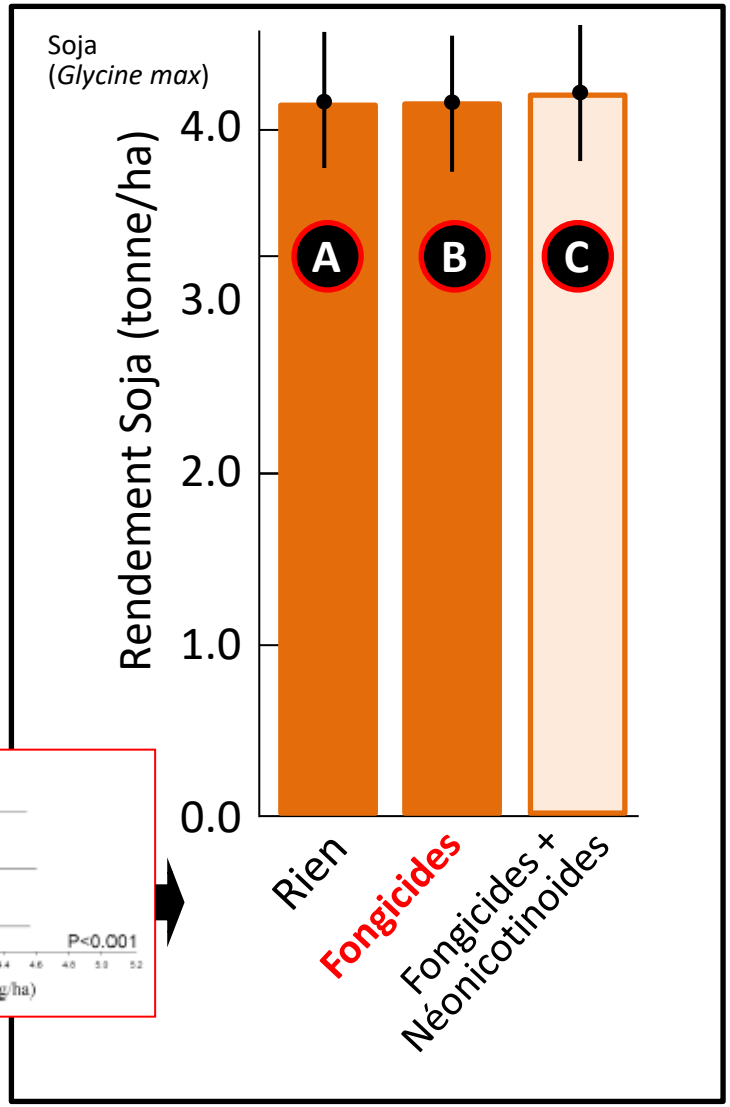


Figure 1. Location of individual experiments that were included in the study. Experiments with the same color belong to a cluster with similar growing environments, as were described in methods and Supplementary Table S1.

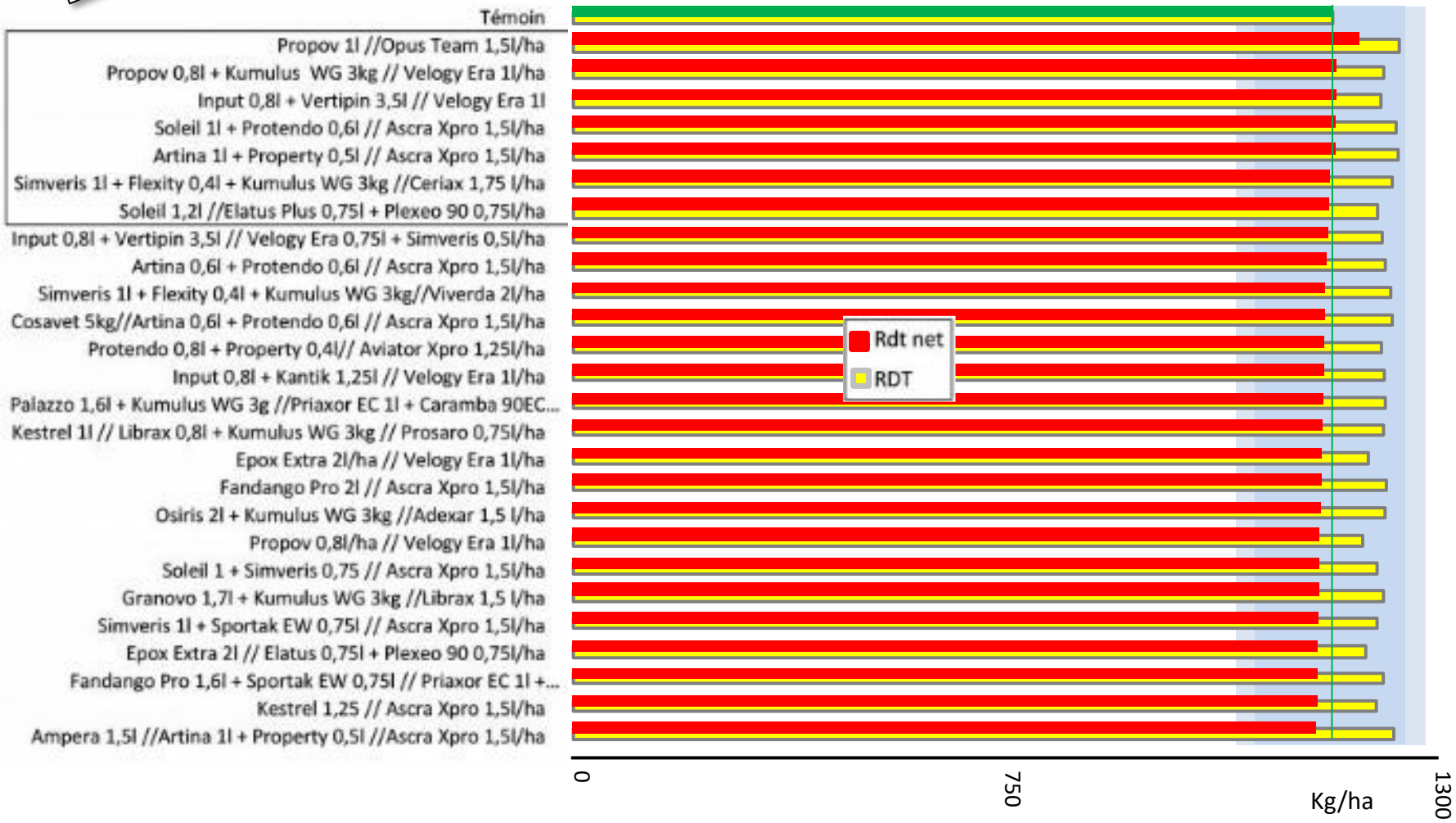
Fig. 2



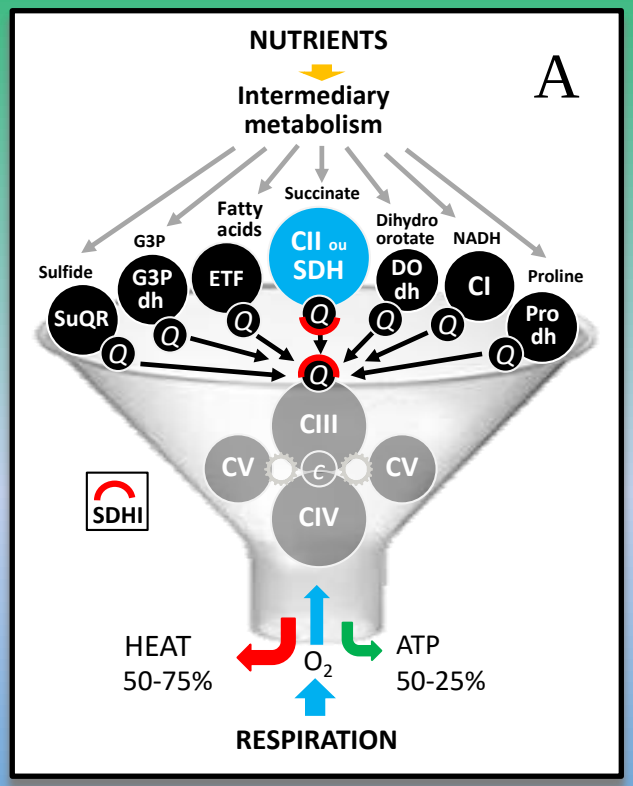


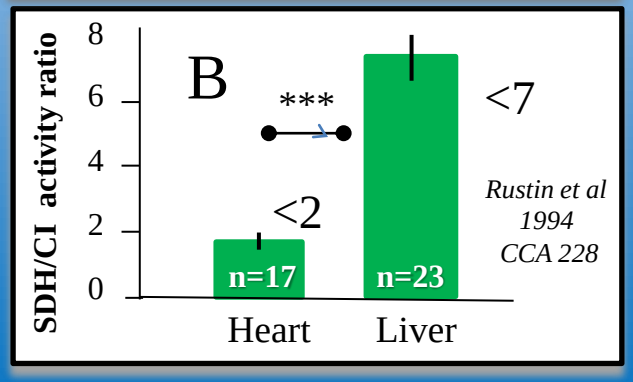
Wheat

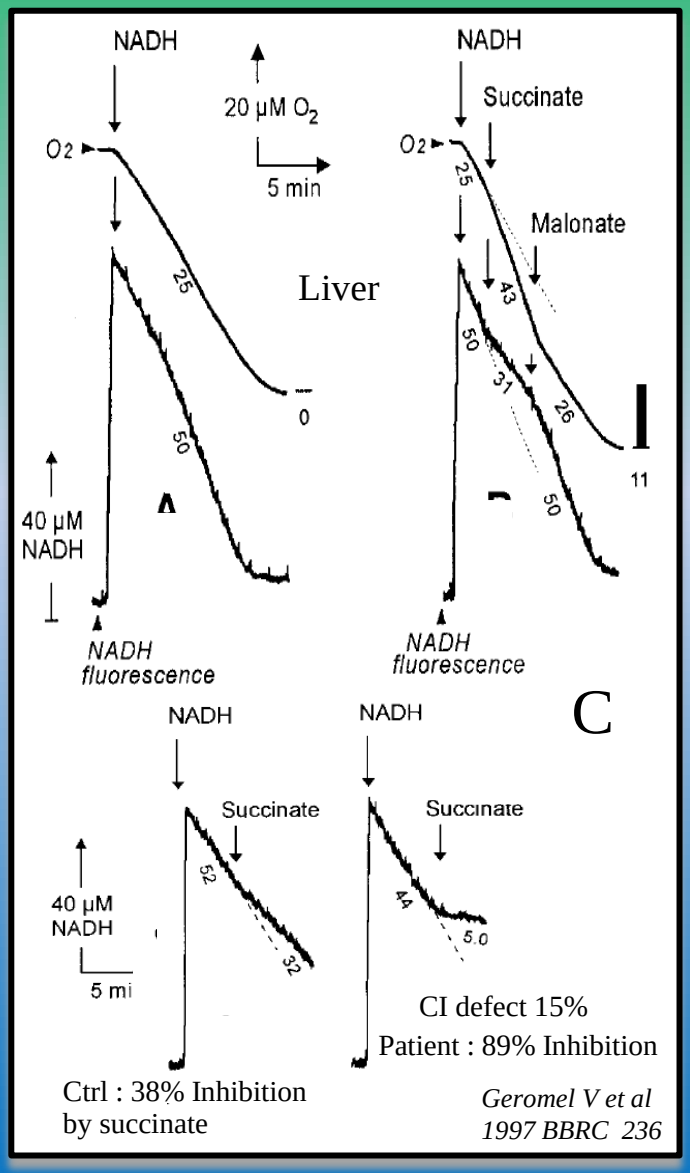
Doubles et triples traitements : Gains de rendement brut et net par rapport au témoin non traité des programmes en 2020 pour un blé à 190€/t



COMPETION REGULATES ELECTRONS FLUXES FROM DEHYDROGENASES





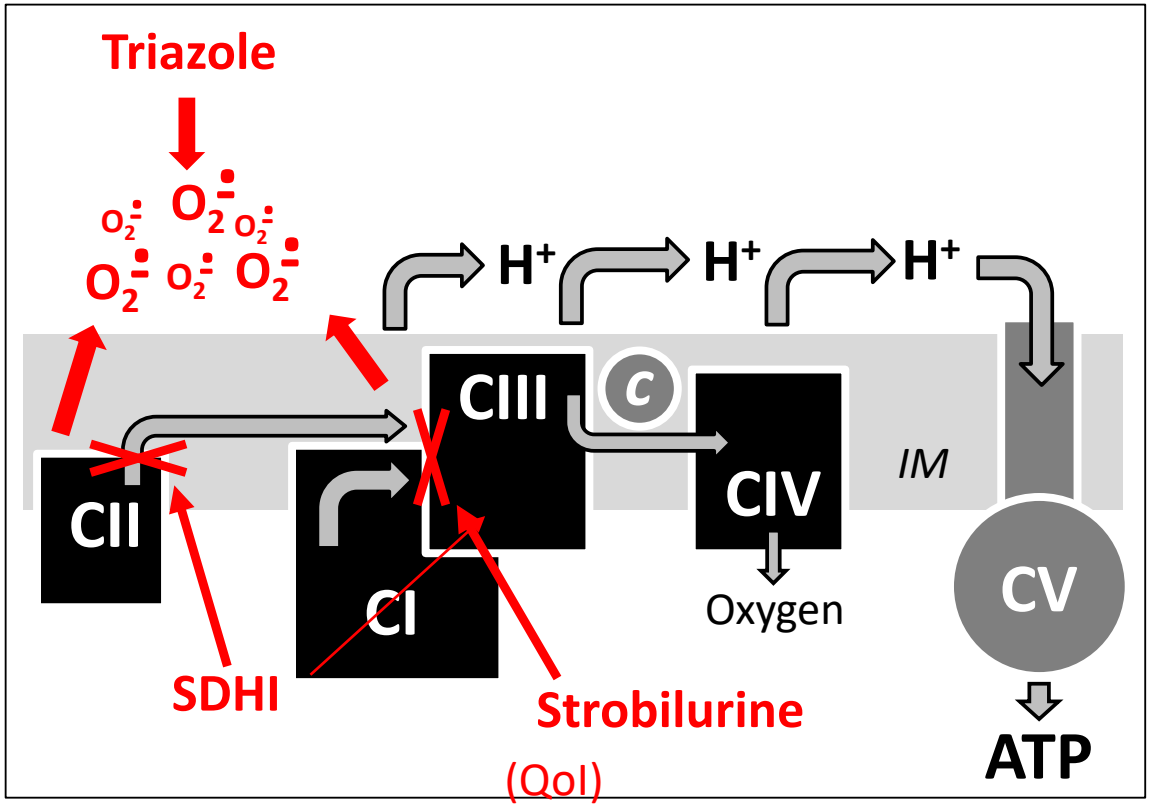


- The respiratory chain regulation is based on competition and cooperation (A) between the dehydrogenases. This regulation also varies from one tissue to another (B)
- The substrates are in competition and a very minimal deficit can cause a very deep blockage (C), an hepatic disease in this example
- Very partial CII blocking, maximum of superoxides produced

Conclusion :

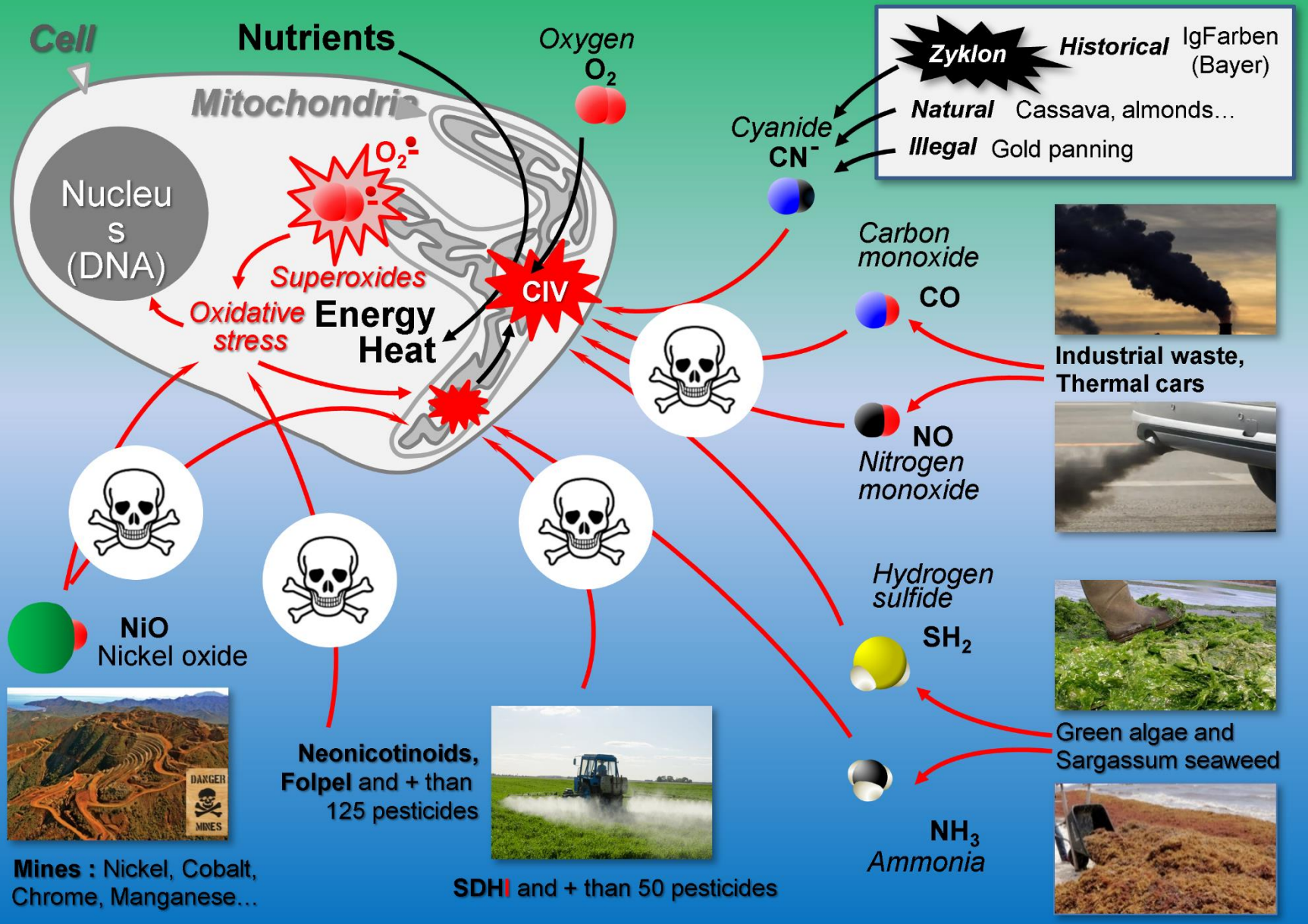
PRECAUTION

Pesticides are tested one by one, but sold as mixtures together with adjuvants



Voxan	Aviator X pro	Keynote	Librax
1 **SDHI**	1 **SDHI**	2 **SDHI**	1 **SDHI**
Fluxapyroxad	*Bixafen*	*Bixafen*	*Fluxapyroxad*
1 **Triazole**	1 **Triazole**	*Fluopyram*	1 **Triazole**
Epoxyconazole	*Prothioconazole*	1 **Triazole**	*Metconazole*
1 **Strobilurine**		*Prothioconazole*	
Piraclostrobine			

Numerous mitochondriotoxicants target cell respiration



Based on scientific evidences PRECAUTION principe

To know more on SDHI

➡ **EndSDHI.com**

« **Le crime est presque parfait** »
Fabrice Nicolino
(Editions: Les Liens qui Libèrent)

Thanks for attention, for earthworms, for honeybees, for birds, and… humans

A magma of skepticism fostered by the blindness of our agencies to scientific evidence surfing on the ignorance of a fraction of the population

Coronaro-sceptics

Bleach, intense light, miracle, Chloroquine, tea…

*USA (**Trump**)*

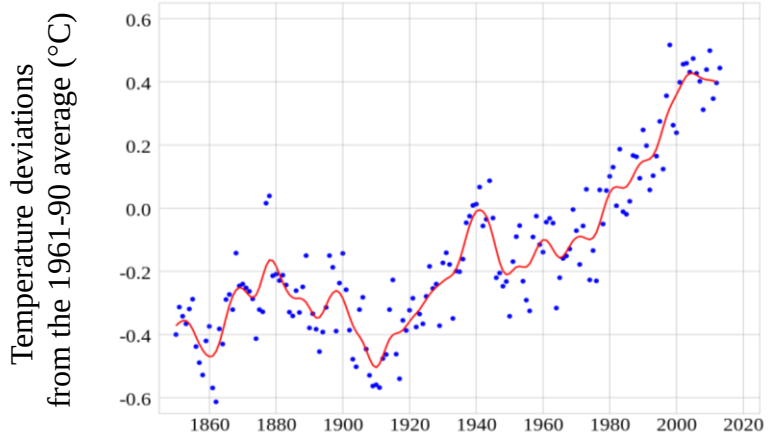
*Brazil (**Bolsonaro**)*

*France (**Raoult**)*

*India (**Modi**)*

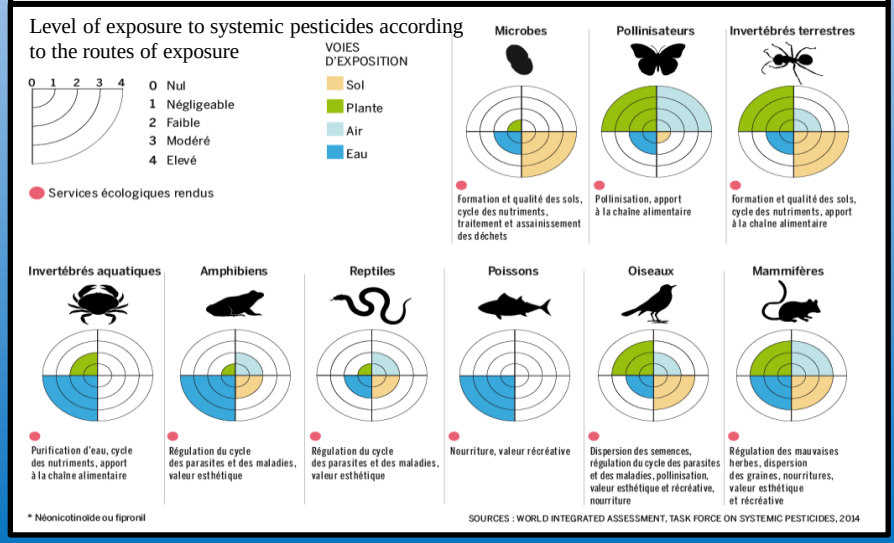
Earthsphero-sceptics

Climato-sceptics

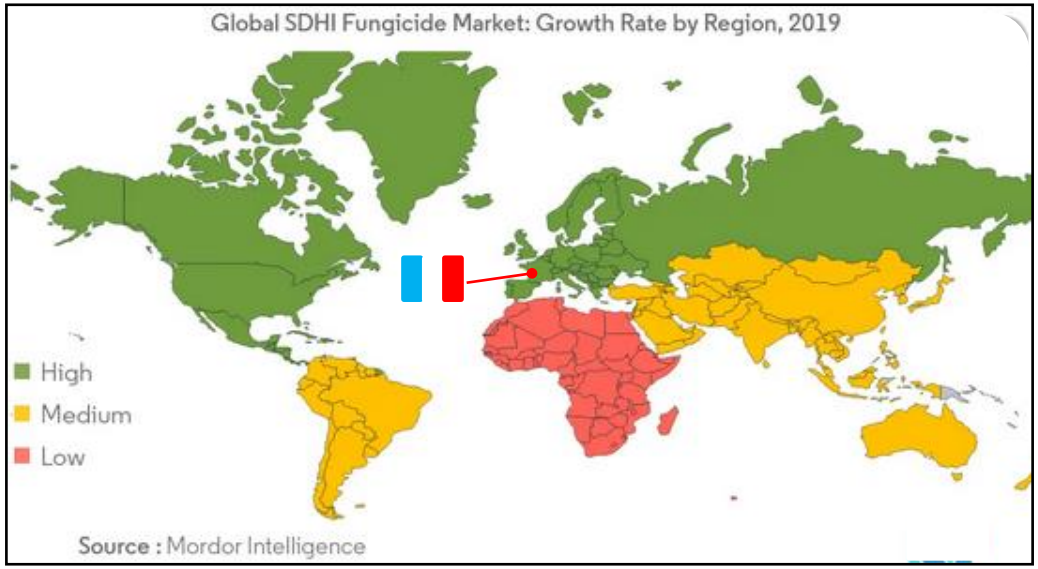


Global surface temperature deviations (1850-2013)

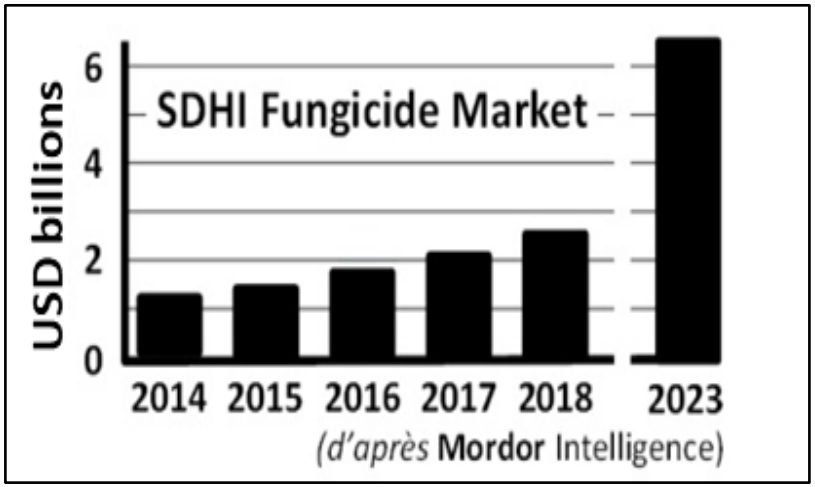
Biodeclino-sceptics & Pesticido-septics



Science against money… is under surveillance!



BASF We create chemistry
CORTEVA agriscience
BAYER
syngenta
UPL



End SDHI : % des consultations par des réseaux identifiables

ANSES	28,2 %
BAYER	**27,8 %**
BASF	**22,5 %**
INSERM	15 %
INRAE	6,4 %

08/09/2020

Inhibition (–) Activation (+) of SDH

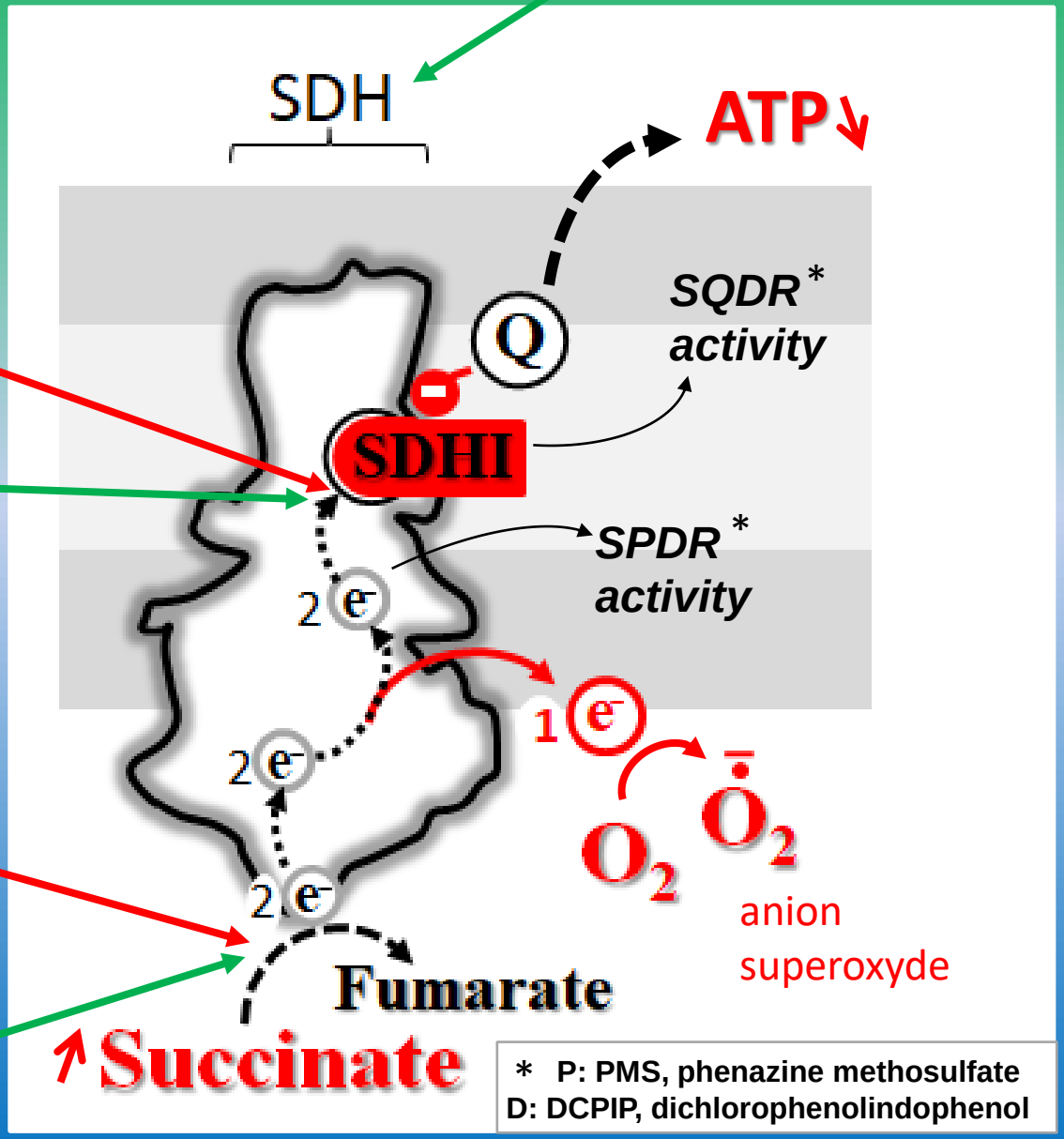
Rapamycin, Naringin boost SDH expression

SDHI
TTFA thenoyltrifluoroacetone
Tocopheryl-succinate
Cyenopyrafen
Cyflumetofen
Pyflubumide
Atpenin 5a –

QH_2 +

Oxaloacetate
Malonate
Dimethyl malonate
Malate
3 Nitropropionate –

ATP +



* P: PMS, phenazine methosulfate
D: DCPIP, dichlorophenolindophenol