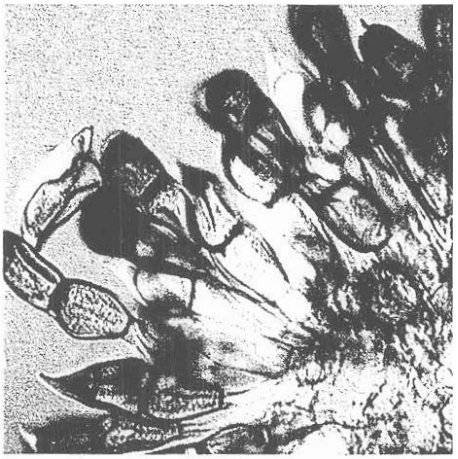
APPENDIX **5**

FLUTRIAFOL



TECHNICAL BULLETIN

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Background

ICI introduced Flutriafol in 1981. Since its introduction the compound has attained an important position in the global fungicide market, where Flutriafol products have proved effective in controlling a vast number of diseases affecting o wide range of crops

In April 2001, Cheminova acquired the global Flutriafol business from Syngenta, including all of the rights, know-how, registrations and trade marks (or the product Today, Cheminova sells the product throughout h e world as a foliar application product for cereals and other amble crops, as a microgranule product for use in coffee ond maize and as a seed treatment product for the control of major seed-borne and soil-borne diseases in cereals. The foliar products are mainly marketed under the well-known trade name Impact® whereas the seed treatment products are sold under the trade name Vincit®.

Flutriafol is an important product in Cheminova's product portfolio and consequently Cheminova will devote considerable effort into supporting the compound in the market.

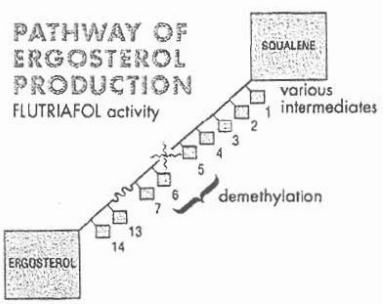
The aim of this technical bulletin is to describe the background to Flutriafol's success and also to inform our partners about h e properties and capabilities of the product.

It will help you to understand the mode of operation, effectiveness and results achieved when using Flutriafol-based products

Discovery and mode of action

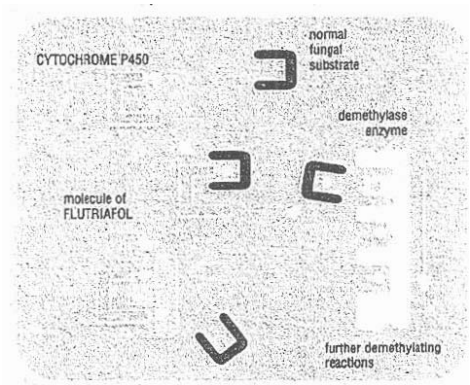
In 1981, scientists at ICI's Jealott's Hill Research Station used computers to model the active binding site on the cytochrome and were able to determine the shape of a fungicide molecule that would best fit. Flutriafol was selected for commercial development as its chemical structure appeared to best suit the modelled binding site.

All azole fungicides a d by blocking the formation of a specific chemical by the fungal pathogen, ergosterol. This blockage occurs in a process called demethylation. Hence azoles are known os Demethylation Inhibitors or DMIs.

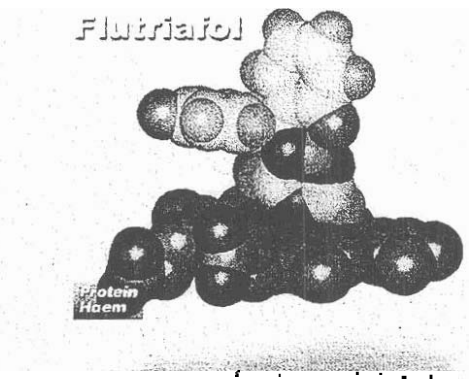


In the demethylation process a protein called Cytochrome P-450 is utilised to bring chemicals together, to be changed and prepared for further demethylation. Flutriafol and other azoles bind to his cytochrome, preventing it from binding to and reacting with the natural chemicals within the fungus

Flutriafol, in common with all other DMIs, has a nitrogen atom that attaches to h e centre of on iron group within the cytochrome, and this prevents the formation of the next intermediate product in ergosterol production.



The stronger h e binding of the fungicide to the cytochrome protein, the better the inhibition of ergosterol production and hence fungicidal activity.



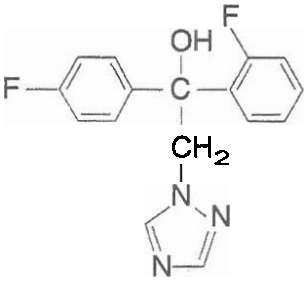
COMPUTER MODEI OF FLUTRIAFOL BOUND ON HAEM (IRON) ff THE CYTOCHROME

CONCLUSION

Flutriafol was found to bind par

Physical/chemical properties of Flutriafol

(RS)-2,4'- difluoro-α-(1H-1,2,4-triazol-1-ylmethyl)- benzhydryl alcohol



common name: **FLUTRIAFOL**

Empirical formula	$C_{16}H_{13}F_2N_3O$
Molecular weight	301.3
Physical state	Solid (crystalline powder)
Colwr	Off-white/light brown
Odour	Odourless
Melting point	130°C
Boiling point	Decomposes
Density	1.41 g/ml at 20°C
Vopour pressure	5.3×10^{-11} mm Hg at 20°C
Solubility in water	130 mg/l at 20°C and pH 7-9
Solubility in organic solvent	Soluble in acetone, methanol, dichloromethane; slightly soluble in xylene
N-octanol/water partition coefficient	Log K_{ow} = 2.29
Flommobility	Not highly flammable
Surface tension	68.7 mN/m at 20 0 ± 2°C (69.7 mg/l flutriafol)
Explosiveness	Not explosive
Oxidising properties	Not oxidising
Storage stability	Stable for at least 5 years at ambient temperature (15-25°C)

Toxicological profile

The acute and chronic toxicity of Flutriafol has been examined in a large number of tests. The major findings are listed below.

Acute toxicity of Flutriafol

Species	Test	Result
Rot (M)	Acute oral	LD_{50}: **1140** mg/kg
Rat (F)	Acute oral	LD_{50}: **1480** mg/kg
Rat	Acute dermal	LD_{50} >1000 mg/kg
Rat	Inhalation (4h)	LC_{50}: 1 **65** mg/l air
Rabbit	Eye irritation	Mildly irritating
Rat/Rabbit	Skin irritation	Nan-irritant
Guinea pig	Sensitisation	Non-sensitiser

Medium and long-term toxicity of Flutriafol

Rat:

3 months feeding study: No Effect Level **20** ppm in diet (approx. **1.5** mg/kg bw/day)

2-yeon feeding study: No Effect Level **20** ppm in diet

Dog:

3 months feeding study: No Effect Level

1year feeding study: **No** Effect Level 1 mg/kg bw/day.

Mutagenicity

Flutriafol is not mutagenic. There was no evidence of mutagenicity in a battery of in vivo and in vitro assays (Ames test, mouse lymphoma mutation assay, rat cytogenic study, unscheduled DNA synthesis, a dominant lethal and a micronucleus study in mice).

Teratogenicity

No teratogenic effects in rats at doses up to **10** mg/kg bw/day and in rabbits in doses up ta 7.5 mg/kg bw/day

Reproduction

In a two-generation reproduction study with rats the reproductive no-effect level was established as **240** ppm

CONCLUSION ON TOXICITY

Flutriafol is classified as slightly hazardous to humans according to WHO classification criteria (WHO Toxicity Class III). Formulated products based on Flutriafol can be handled safely when label recommendations concerning use of personal protective equipment are followed.

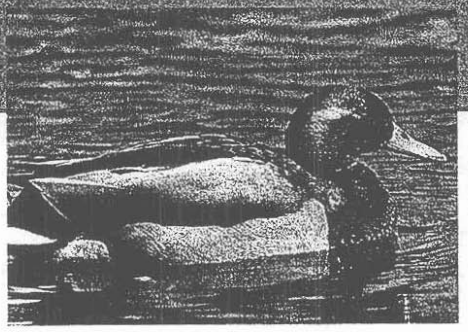
Ecotoxicological properties of Flutriafol

The toxicity of Flutriafol to the fauna has been tested in a range of tests.

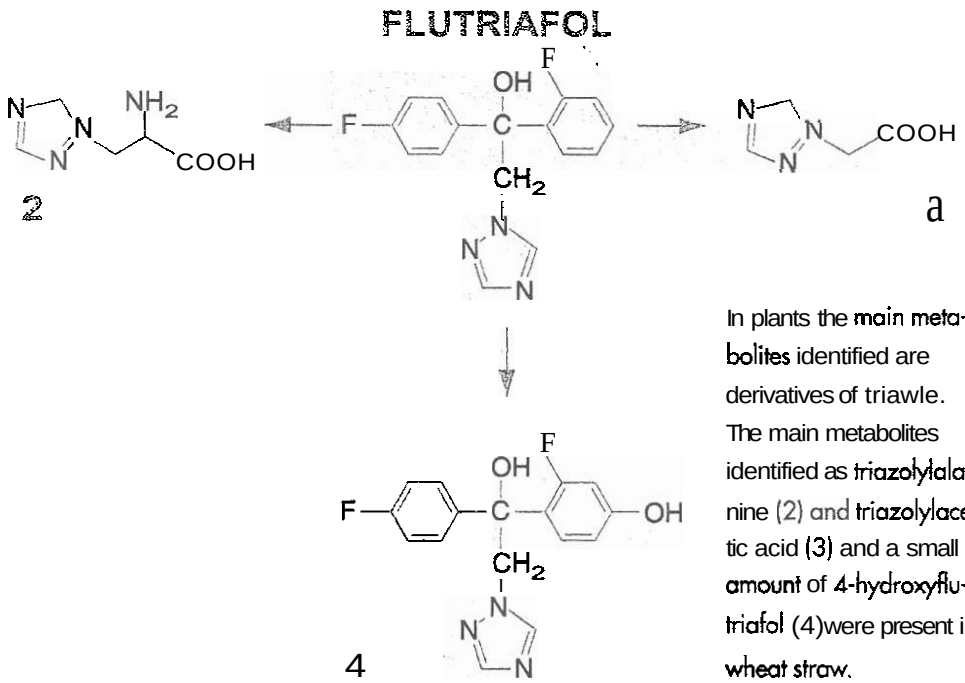
	Species	Test	Result
Birds	Mallard duck	Acute oral	LD_{50}: >5000 mg/kg
	Red-legged partridge	Acute oral	LD_{50}: 616 mg/kg
	Mallard duck	Dietary	LC_{50}: 3935 ppm
	Bobwhite quail	Dietary	LC_{50}: 6352 ppm
	Japanese quail	Dietary	LC_{50}: 17083 ppm
Aquatic organisms	Rainbow trout	96 h	LC_{50}: 61 mg/l
	Mirror carp	96h	LC_{50}: 77 mg/l
	Daphnia	48 h	EC_{50}: 78 mg/l
	Algae	96 h	EC_{50} (growth inhibition):12 mg/l
Other non target organisms	Tested on formulation: Earthworm Eisenia foetida	14 doys	LC_{50}: >1000 mg/kg
	Bees	48 h	LD_{50} [oral and contact): >50 mg/bee

CONCLUSION OM ECOTOXICITY

The ecotoxicological profile of Flutriafol demonstrates, that Flutriafol presents a low risk to the environment when handled according to label recommendations. Flutriafol has low toxicity to most wildlife species tested. Flutriafol is classified as harmful to aquatic organisms (EU classification), for which reason release to this environment should be avoided.



Metabolism in plants



In plants the main metabolites identified are derivatives of triawle. The main metabolites identified as triazolylalanine (2) and triazolylacetic acid (3) and a small amount of 4-hydroxyflutriafol (4)were present in wheat straw.

Analytical methods for residues

Residues can be analysed by various methods, including extraction using acetonitrile and water, and qualitatively and quantitatively determined by GLC.

Fate in soil

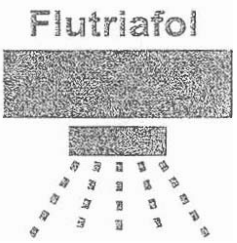
Flutriafol degrades slowly in soil with a half life of more thon one year. No significant degradation prod. ucts have been identified. It hos been demonstrated in field studies, that residues in soil do not infer phytotoxicity effects on following crops Flutriafol is moderately mobile in soil. Field studies have indicated that there is no particular concern for leaching to ground water under practical use conditions

Biological properties

Many factors can influence the activity of Flutriafol Systemic fungicides (like Flutriafol) differ from protectants. The latter have to form a protective layer on the plant surfaces

Systemic fungicides hove to overcome extra barriers before coming into contact with h e target organism.

In addition to the intrinsic toxicity of Flutriafol to the target pathogen, its pattern of uptake and movement within the plant is critical.



Technical influences		Atmospheric influences1 Influence of crop
Formulation Drop veiocity	**Drop deposition**	Angle of plant surface Movement of plant Wax layer
Formulation Active ingredient	**Penetration in the plant**	Wax cuticuia
Formulation Active ingredient	**Systemic transport**	Membrane structure
Formulation Active ingredient	**Penetration in the fungal cells**	Fungal cell wall
Active ingredient	**Fungicidal activity**	Site of action

Contact fungicides

The ease with which fungicides enter and move within the plant is largely determined by their physical and chemiml properties.

One measure of this is the ability of the fungicide to distribute itself between alcohol (octanol) and water when shaken up in a mixture of the two substances. This is called its Partition Coefficient or Log K_{ow} value.

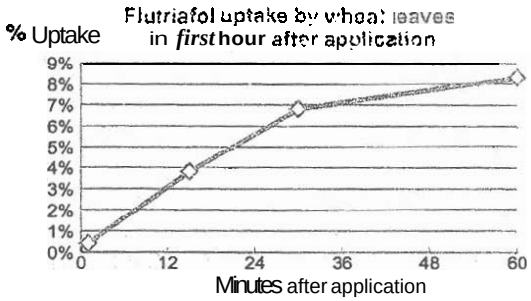
Fungicides with a value of *3.2* or less all move fast within cereal plants. Those with higher values do not move very rapidly, although they m n penetrate the plant.

Log K_{ow} values of different products

Product	Log K_{ow} Values
Flutriafol	2.3
Cyproconazole	2.9
Triadimenol	3.2
Tebuconazole	*3.7*
Propiconazole	*3.8*
Hexaconazole	*3.9*
Flusilazole	3.9

The low Log K_{ow} value of Flutriafol allows it to rapidly penetrate through h e waxy outer layer of the cereal leaf [he cuticle).

This is especially so over the first 24 hours after application. In fact, depending on conditions, Flutriafol often penetrates very rapidly even during the first hour



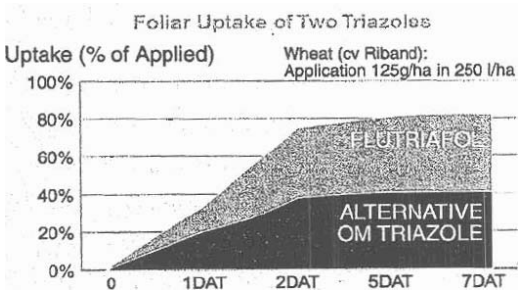
(First how uptake of Flutriafol using radio labelled C14 Flutriafol recovery as % of applied.)

As the spray droplets dry on the leaf the concentration of the active ingredient increases. Most uptake occurs during the latter part of this drying process.

Generally speaking with natural droplet drying, uptake over h e first 24 hours can be around 35-45 per cent of that applied on a leaf.

Radio-chemical studies undertaken at Jealott's Hill Research Station illustrate the uptake of Flutriafol over a seven-day period compared to another manufacturers (OM) triazole.

Within three days of application about 80%of the applied Flutriafol has been absorbed.

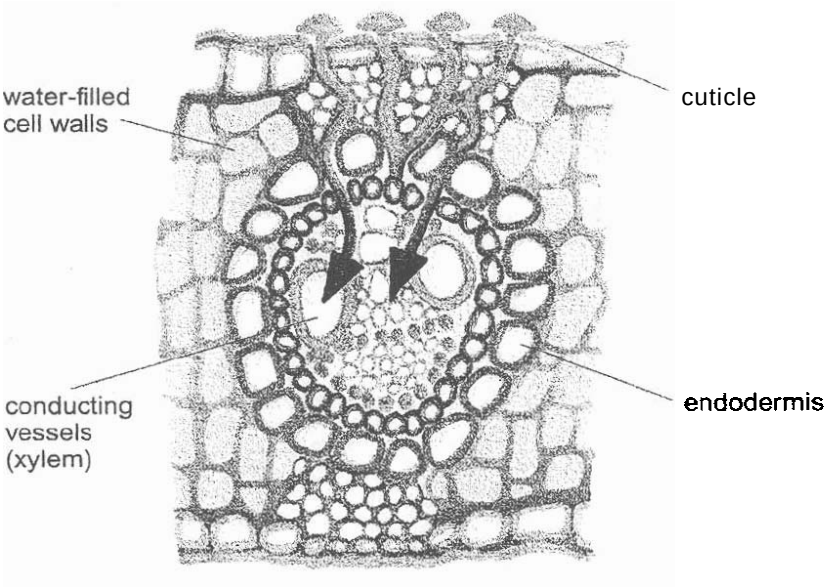


Once inside the plant Flutriafol does not accumulate ot the point of entry, but diffuses into the underlying tissues. Some of the Flutriafol will move into the water-filled cell walls and may move up the plant in the transpiration stream in a network of cell wall interconnections.

However, most of the Flutriafol is transported up the plant (acropetal movement) in the main conducting tissue – h e xylem. To do this it has to cross another formidable cell boundary called the endodermis. Again, the physical/chemical properties of Flutriafol allows this with relative ease.

There is no evidence of Flutriafol moving in the phloem.

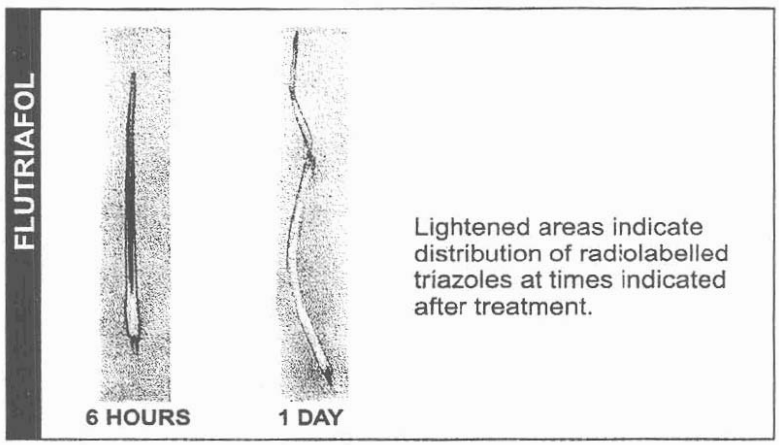
Cross section of cereal leaf

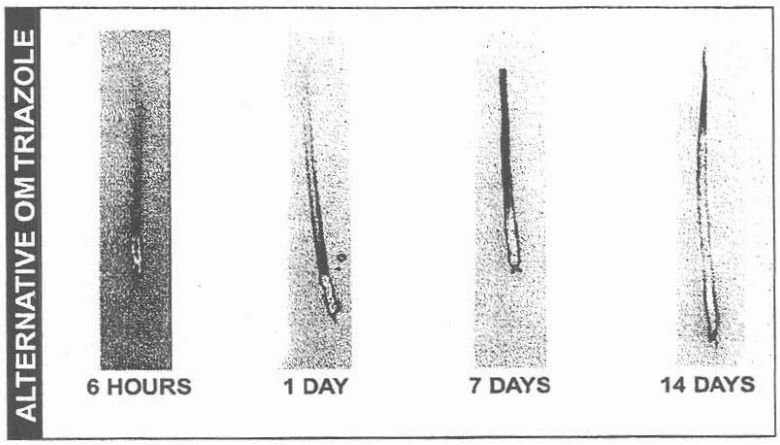


Studies by Jealott's Hill scientists using radiolabelled Flutriafol show the movement of the product visually from a leaf base application, with time.

Autoradiographs identify Flutriafol as a highly systemic product. Within 24 hours Flutriafol redistributes throughout the entire leaf several times foster than other triazoles.

Single leaf autoradiographs (C^{14} radiolabelled Flutriafol)





CONCLUSION ON SYSTEMICITY

>>> Flutriafol is clearly one of the most mobile of the modern triazoles.

>>> Flutriafol moves rapidly to protect new leaves not unfurled at time of application – this is particularly important in a fast-growing crop such as wheat.

>>> Flutriafol moves rapidly to sites of established infections for fast curative activity.

>>> Where spray coverage is less than optimal (often with lower volumes at flag leaf) Flutriafol redistribution ensures that vital leaves are not left unprotected.

>>> With products of slower redistribution there is a higher risk of disease establishing in new or unprotected growth. The disease is then more difficult to control once the product finally reaches these areas.

Flutriafol products are therefore effective both before and at the onset of infection.

Diseases controlled

Flutriafol *is effective* against *most* pathogens *belonging to the* asco- *and* basidiomycetes class of *fungi.*

Apples
Podosphora leucotricha
Venturia inaequalis
Bananas
Mycosphaerella fijicola
Mycosphaerella musicola
Cereals
Erysiphe graminis
Helminthosporium spp.,
Puccinia spp.
Rhynchosporium secalis
Septoria spp.,
Tilletia spp.,
Ustilago spp.,
Chicory
Erysiphe cichoracearum
Puccinia cichorii.,
Coffee
Hemileia vasatrix
Corn
Sphacelotheca reiliana
Flax
Colletotrichum lini
Grapevine
Guignardia bidwelli
Uncinula necator
Peas and beans
Aschochyta pisi
Uromyces pisi
Colletotrichum pisi
Peanuts
Mycosphaerello arachidis
Mycosphaerella berkeleyi
Puccinia arachidis
Oilseed rape
Alternaria spp.
Cylindrosporium concentricum
Phoma lingam
Potatoes
Alternaria solani
Soybeans
Cercospora kikuchii
Cercospora sojiana
Michrosphaerea diffusa
Septoria glycines
Sugar beet
Cercospora beticola
Erysiphe betae
Ramularia beticolae
Uromyces betae
Sunflowers
Diporthe heliinthi
Erysiphe spp.,
Cercospora spp.,

Crop-wise usage in various countries

Flutriafol is today registered in more than 50 countries throughout the world as a folior as well as a seed treatment product.

To illustrate the considerable wriotion in the use of Flutriafol in different countries, some of its crop outlets are listed below

Argentina
Cereals, potatoes, peanuts
Australia
Cereals, oilseed rape
Brazil
Cereals, coffee
France
Cereals, chicory, maize, oilseed rape, peas, sugar beet
Germany
Cereals, maize
Italy
Cereals, sugar beet
Russia
Apples, cereals, grapevine, sugar beet, sunflower

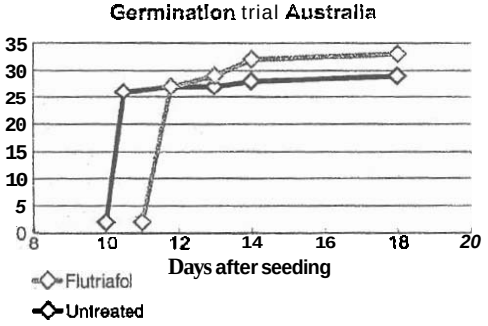
Selectivity

All triazoles generally interfere with the synthesis of gibberellins in many craps whereby a growth-regulating effect can be seen.

Compared to other triazoles, the growth-regulating effect of Flutriafol is law.

The growth-regulating effect is particularly critical when triazole fungicides are used for the treatment of seeds.

A large number of trials hove been conducted in order to establish that Flutriafol con be used safely as a seed treatment product. The general conclusion of the trials is that Flutriafol delays emergence, but after four weeks, no difference can be observed.



The delay in emergence increases at lower temperatures. Lower dose rates are therefore tolerated in colder climates.

Dosage rates up to 50 ppm in wheat and 150 ppm in barley ore generally recommended as safe to use independent of the weather following application.

Anti-resistance strategy

According to the FRAC (Fungicide Resistance Action Committee), h e resistance risk of using triazole fungicides is moderate.

It is recommended to either apply Flutriafol in mixture or in sequence with fungicides with another mode of action. The most important ore chlorothalonil, benzimidazoles, morpholines and strobilurines.

Mixtures

Flutriofol is today marketed in mixtures with:
Chlorothalonil
Carbendazim
Thiabendazole
Imazalil

And the insecticide
Carbofuran

DISCLAIMER

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