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Prediction of the microbial biodegradative pathway of organophosphorus pesticide Chlorpyrifos using the web based open access pathway prediction system of the University of Minnesota's Biocatalysis/ Biodegradation database

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ABSTRACT: The ability to predict biodegradation of toxic compounds whose biodegradation has not yet been fully studied have many practical implications that help researchers to know the harmful effects of long term environmental exposure to these compounds in relation to human health and the environment. The present study was therefore undertaken to predict the biodegradation of one of the most widely used toxic organophosphorus pesticide, Chlorpyrifos, using a web based open access pathway prediction system available in the University of Minnesota's biocatalysis/biodegradation database. The reconstructed general pathway of biodegradation of the pesticide had high similarity to the reports of the microbial biodegradative pathway of the same compound studied through *in-vitro* and *in-vivo* models. Such web based open access systems for predicting the biodegradation of toxic pesticides and related nerve agents will therefore serve as a boon for the environmental regulatory authorities and researchers especially in the developing nations, in years to come.

KEYWORDS: Prediction, biodegradation pathway, microbial, organophosphorus, chlorpyrifos, open-access, database.

INTRODUCTION

The ability to predict biodegradation pathways accurately has practical implications that go beyond functional genomics. Industry will continue to synthesize new materials faster than it, or regulatory agencies and academic researchers, can study their environmental fate. Many companies already invest significant resources to predict the biodegradation pathways of their new compounds. This avoids the commercialization of materials that are later found to be dangerous and thus need to be withdrawn from commerce. Through such efforts, companies save the capital expended in developing an unusable compound and mitigate against the negative publicity engendered for marketing an environmentally unsound product. Thus, the improved accuracy of industrial biodegradation predictions can benefit both individual companies and society as a whole.

As less than 0.01% of known organic compounds have been tested for biodegradation, predictive methods become the only recourse to avoid situations in which people are exposed to known carcinogens for years before action is taken. (Wackett and Ellis, 1999).

Chlorpyrifos is one of the world's most widely used organophosphorus pesticides in agriculture. Exposure to chlorpyrifos and its metabolites have been related to a variety of nerve disorders in humans. Chlorpyrifos, which was previously thought to be immune to enhanced biodegradation, has now been shown to undergo enhanced biodegradation by bacterial and fungal species (Bhagobaty *et al.*, 2007).

Although an attempt has been made to propose a possible pathway for chlorpyrifos degradation by microorganisms using the available scientific reports (Singh and Walker, 2006), its exact microbial biodegradative pathway has not been elucidated till date. The present article therefore aims at predicting a possible a microbially mediated biodegradative pathway for this toxic organophosphorus pesticide using an open access, web based biodegradative pathway prediction system available in the University of Minnesota's Biodegradation database.

METHODOLOGY

All experiments were carried out in the web based pathway prediction system (PPS) (http://umbbd.ahc.umn.edu/predict/) available in the University of Minnesota's Biocatalysis/Biodegradation database (UMBBD). The SMILES code used for generating the Javascript image of Chlorpyrifos in the PPS was CCOP (=S) (OCC) Oc1nc (Cl) c (Cl) cc1Cl (Source http://en.wikipedia.org/wiki/Chlorpyrifos). The figures generated were arranged to make a possible biodegradative pathway for chlorpyrifos using the metabolic logic of the pathway prediction system and the available rules and datasets in the UMBBD database. The biotransformation (bt) rules used by the PPS in generating the chemical structures of the pathway are enlisted in Table 1.The complete list of all the bt rules are directly accessible from the UMBB database.

Table 1: List of all biotransformation rules used by the pathway prediction system (PPS) to generate the pathway of biodegradation of Chlorpyrifos.

Biotransformation (bt) rule as in the UMBB database	The chemical reaction
bt0102	Aromatic thiophosphate ? Hydroxyaromatic derivative + Thiophosphate derivative
bt0029	OrganoHalide ? RH
bt0362	2- or 3-Carboxypyridine ? 2, 5-Dihydroxypyridine
bt0363	2, 5-Dihydroxypyridine ? Pyruvate
bt0103	Thiophosphate diester ? Phosphate diester
bt0361	Aliphatic Phosphodiester ? Alcohol + aliphatic Phosphoester
bt0361	Aliphatic Phosphoester ? Alcohol

RESULTS AND DISCUSSION

Our results show that chlorpyrifos (0, 0-diethyl 0-(3, 5, 6-trichloro-2-pyridyl) phosphorothioate) is degraded by microbial metabolism primarily in the aerobic mode to two major groups of chemicals structures (Figure 1). The ring portion of chlorpyrifos is catabolised in a separate pathway that finally yields pyruvate

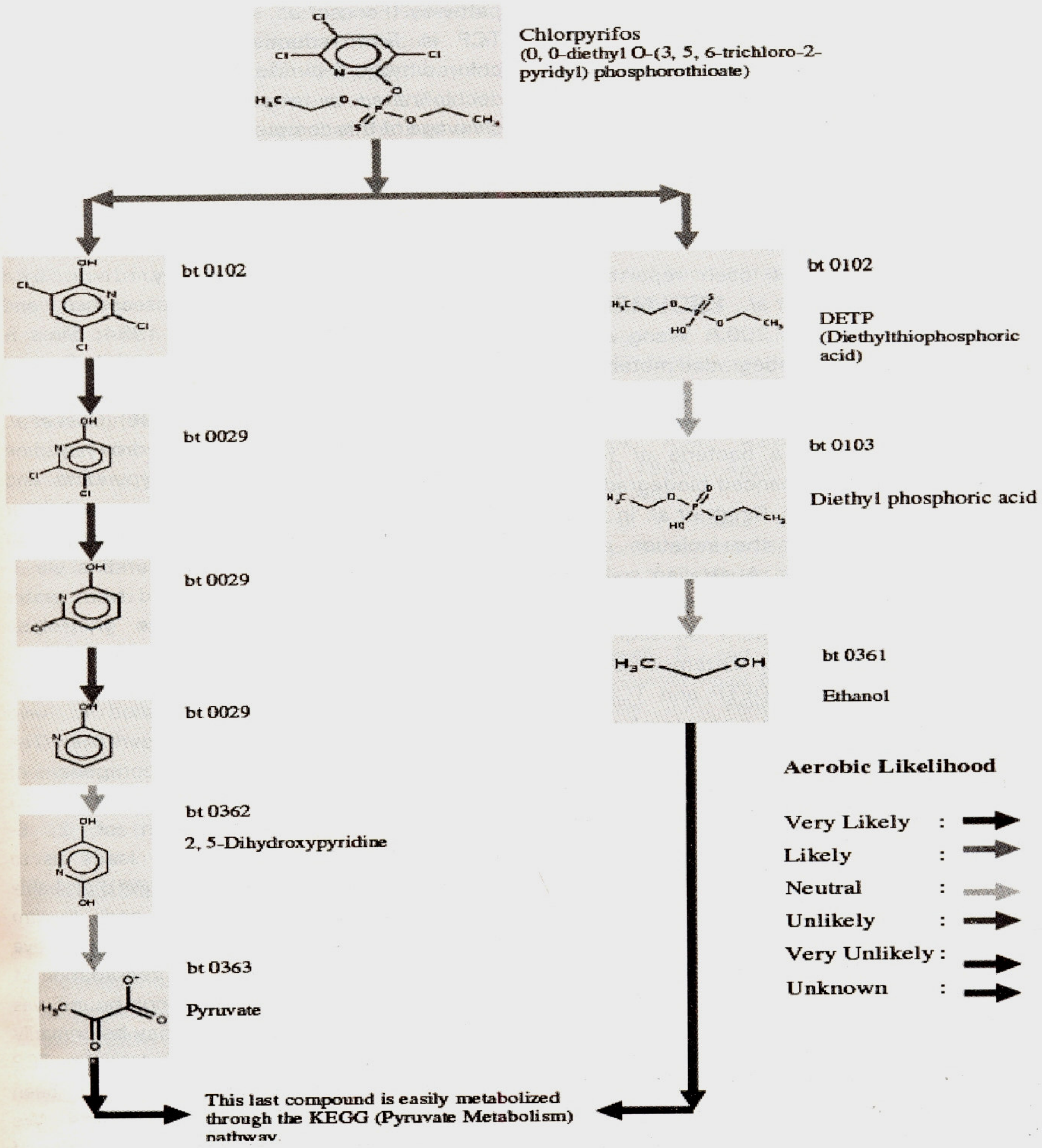

Fig.1: The reconstructed pathway of microbial degradation of Chlorpyrifos using the pathway prediction results of the pathway prediction system (PPS) in the UMBB database.

as the major end product. Pyruvate is then subsequently degraded through the KEGG pathway. The other side chain of the parent molecule is predicted to be biotransformed into diethylthiophosphoric acid (DETP). DETP is finally catabolised to ethanol, from which it is subsequently taken up by the pyruvate pathway (KEGG) .The degradation of chlorpyrifos to yield DETP and TCP (3,5,6-trichloro-2-pyridinol), has been reported by many workers (Richnis *et al.*, 1997; Mallick *et al.*, 1999; Horne *et al.*, 2002; Wang *et al.*, 2002). All these major biodegraded metabolites were thought to be immune to microbial action and this was provided as a significant reason for the inability to isolate a bacteria or fungal species that showed enhanced biodegradation of chlorpyrifos. However, Singh *et al.* in 2003 was the first to report the isolation of an *Enterobacter sp*. from an Australian soil that exhibited enhanced biodegradation of chlorpyrifos in culture medium. Subsequently, it was shown that the *Enterobacter sp*. degraded chlorpyrifos to DETP and TCP and was able to utilize DETP as a source of carbon and phosphorous for its growth (Singh *et al.,* 2004). A strain of *Pseudomonas acidovorans* was also reported to be able to use DETP as a sole source of sulfur (Cook *et al.,* 1980). Our *in silico* pathway prediction results relating to the degradation of DETP are therefore in general agreement with the results obtained by various workers worldwide using a cultivation dependent approach for detecting the degradation of chlorpyrifos.

Feng *et al.,* (1997) isolated a *Pseudomonas* sp. which can mineralize TCP in liquid medium. Later, on the basis of combined experiments with photolysis and microbial degradation, suggested that TCP was metabolized by a *Pseudomonas* sp. by a reductive dechlorination pathway (Feng *et al.,* 1998). In this pathway, TCP is first reductively dechlorinated into chlorodihydro-2-pyridone, which is further dechlorinated to tetrahydro- 2-pyridone. Ring cleavage of this compound resulted in formation of maleamide semialdehyde, which is metabolized to water, carbon dioxide and ammonium ions.

Microbial degradation of analogous compounds such as pyridine and hydroxypyridine has been researched and reviewed extensively (Shukla, 1984; Sims & O'Loughlin, 1989; Kaiser *et al.,* 1996). Several microorganisms were reported to degrade hydroxypyridine (Kaiser *et al.,* 1996). Cain *et al.* (1974) reported that 2- or 3-hydroxypyridine was oxidized to 2, 5-dihydroxypyridine and production of maleamic acid occurred later through ring cleavage. Oxygen atoms used to transform 4-hydroxypyrhydroxypyridine via 3, 4-dihydroxypyridine were derived from water molecules by hydroxypyridine hydrolase (Watson *et al.*, 1974). It is likely that TCP is metabolized in a similar manner, as one of the metabolites of TCP was identified to have similar structure to 2-hydroxypyridine. The pathway predicted for the ring component of the chemical structure of chlorpyrifos (Figure 1.) also leads to the generation of 2, 5-dihydroxypyridine and therefore leads us to believe that the predicted pathway is a probable process in the real *in-vivo* and *in-vitro* conditions. However, the present degradative pathway failed to predict the degradation of chlorpyrifos to TCP as no such compound was predicted in the pathway. This may be primarily because of the fact that the data for TCP biodegradation by bacteria has not been incorporated into the UMBB database. We therefore, tried to undertake the degradation of TCP separately (results not shown) in the PPS but failed to generate a possible aerobic degradative pathway for the same. However, degradation of the parent molecule i.e. chlorpyrifos yielded a similar likely pathway for the degradation of the ring component (Fig. 1). However, it has to be noted that the pathway prediction system available in the UMBB database uses the available biodegradation data submitted to it by various research workers worldwide and therefore accuracy in its prediction may deviate from the actual phenomenon due to some data gaps caused as a result of non–incorporation of the microbial biodegradative data for chlorpyrifos and its metabolites into the database.

The significance of our exercise, lies in the fact that the metabolic logic of a web based open-access pathway prediction system has been proven to give a similar picture of the degradative mechanism involved in the microbial catabolism of a toxic molecule that is widely used in the agricultural practices of developing countries like India. Pathway prediction systems, such as these, can and will therefore be widely relied upon in the near future to predict biodegradation of similar newly synthesized compounds. This saves time and energy on the part of the researchers, to know the final ecological and medical implications of long term exposure to such toxic compounds in the environment.

CONCLUSIONS

Our results clearly indicate, that the microbial degradative pathway for the toxic organophosphorus pesticide, chlorpyrifos, can be predicted using the available data in the open access pathway prediction system of the University of Minnesota's biocatalysis/ biodegradation database to a very high level of similarity to the actual biodegradative pathway predicted on the basis of experimental analysis of the phenomenon. As research in the degradation of this toxic pesticide progresses further and the genetic basis of its microbial metabolism is found out, we hope that the pathway prediction system of the UMBB database will also evolve to give a much higher accuracy of prediction for the biodegradative pathway of chlorpyrifos and other related organophosphorous pesticides and nerve agents for which biodegradation studies are few or have not been initiated.

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