ASSESSMENT OF THE POTENTIAL RISKS TO MICROBIOTA FROM LOW DOSE DECHLORANE PLUS EXPOSURE IN THE SOIL MIMIC CONDITION

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Introduction

Dechlorane plus (DP-$C_{18}H_{12}Cl_{12}$), a high chlorinated flame retardant, is listed as a high production volume (HPV) chemical. The technical DP product consists of two isomers: *syn* and *anti* in a ratio 1:3 and it is used in electrical hard plastic connectors in televisions and computer monitors, wire coatings, and furniture[1,2].As regards environmental concern, DP is considered as low bioaccumulation risk compound because of high logK_{ow} (~ 9.03) but the long haft-life of DP in soil and water (8640 h and 4320 h, respectively[1]) could raise a big concern to the ecosystem. The environmental occurrence of two stereoisomers *syn* and *anti* was first investigated from the Great Lakes region by Hohnet al.[3]. Although both isomers can bio-accumulate, there are different potentials to their bio-magnification in aquatic food webs[5].Tomy's research group[4] had reported that the anti-isomer was detected dominantly in higher trophic level (TL) organisms like walleye and goldeye while the *syn*-isomer was found mainly in the lower TL organisms like zooplankton and mussels. In addition, the different solubility of two isomers was mentioned in the Report of Oxygen (2008)[6] at concentration of 207 and 572 ng/L. That characteristic implied that these two isomers could exhibit different behaviors and thus have different environmental effects, especially in the food chain and ecological communities[7].

In our study, we evaluated the effects of DP on bioactivities in the soil mimic condition. By using various bacteria which play specific roles in ecosystem, we could predict the potential interaction among two isomers and biota, especially the microbial activities. This is the first report about the fates of DP on pure bacterial cultures.

Materials and Methods

Microorganisms

Three types of bacteria were obtained in this study: *Escherichia coli* (*E.coli*); dibenzofuran (DF) degrading-bacteria *Agrobacterium* sp.PH-08 (Gen Bank Accession numbers: JN862809) isolated from contaminated soil by enrichment cultures using DF as the sole carbon and energy source; and phosphorus solubilizing bacteria *Gluconacetobacterliquefaciens* sp. G1 isolated from the leaves of Bamboo at Miryangby Applied and Environmental Microbiology laboratory (Pusan National University, Korea).

Effects of DP isomers on bacterial cell viability

All bacterial species were collected at stationary phase. Resting cells were prepared and kept stably in PBS buffer pH 7.4 for 1 h. A certain amount of each bacteria was added into PBS solution and soil extracted medium containing 250 ppb of each DP isomer (Wellington Laboratories)to the final concentration around 10^6 – 10^7 CFU/ ml.The cell viability was estimated at 0, 3, 6, 9 and 24 h using CFU counting. In addition, the Propidiun Iodine (PI-Sigma) staining was performed to confirm the number of dead bacteria after 2 h treatment.

Estimating the induced intracellular ROS level in DP treatment

Intracellular ROS level was evaluated using 2'-7'-Dichlorodihydrofluorescein diacetate (DCFH-DA – Sigma) assay. DP treated bacteria were collected by centrifugation after 2 h incubation and resuspended in 1ml PBS buffer pH 7.4. 10 ul of DCFH-DA 5mM was added into each solution and the fluorescent intensity was measured at excited wavelength 488 nm.

Inhibition activities of DP exposure bacteria

In our study, we estimated the Phosphorus solubilizing capacity of *Gluconacetobacterliquefaciens* sp. G1 and Dibenzofuran degrading ability of *Agrobacterium* sp. PH-08. G1 was grown in NBRIY medium containing 1 g/L

insoluble $Ca_3(PO_4)$. The soluble phosphate concentration was determined using Phosphomolybdate method[8]. DF-degrading activity of PH-08 grown in MSM medium with 5 mM DF as the carbon source was evaluated via remaining DF concentration using HPLC analysis (60% ACN + 40% H_3PO_4 0.1%).

Results and discussion

Effects of DP isomers on bacterial cell viability

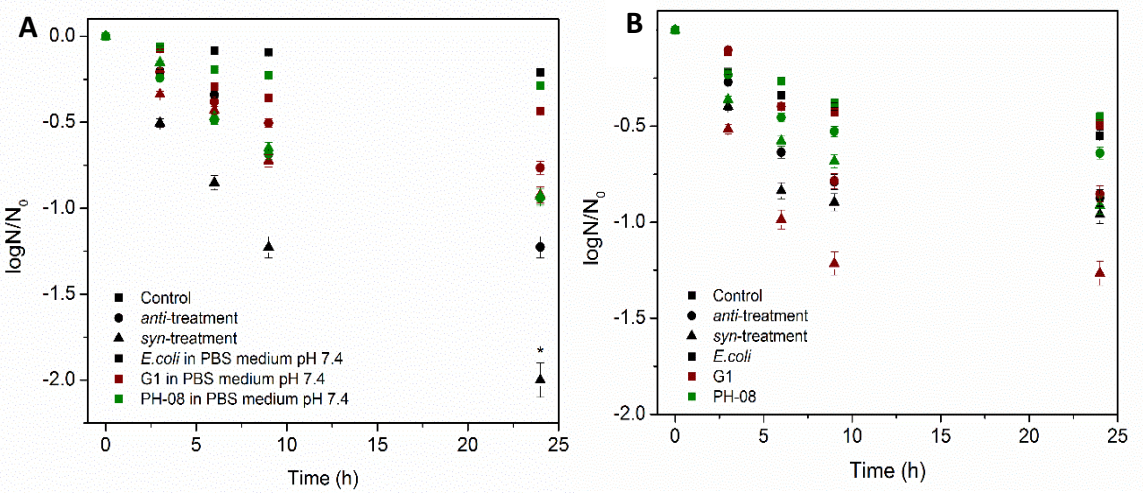

*Figure 1: CFU value of E.coli, G1 and PH-08 under exposure of two DP isomers in PBS medium (**A**) and soil extraction (**B**). * means not detected*

The CFU in *syn*-DP treatments showed the lowest value in the PBS or soil extracted medium (Fig. 1). Especially, the inhibition of CFU was significantly observed in *E.coli* and PH-08 samples(from 78%-94% in PBS medium after 9 h DP exposure). The decreasing CFU of G1 and PH-08 could not be evaluated after 24 h treatment as compared to 9 h. On the other hand, the one of *E.coli* still was decreased up to 91% and undetectable level in *anti*- and *syn*- treatments, respectively. In soil extracted medium, the controlswere decreased rapidly up to 72%, 68.8% and 64% in*E.coli*, G1 and PH-08 samples respectively. In the soil system, the different effect of *anti*- and *syn*-isomers could be neglected while the G1 and PH-08 samples could show the higher inhibition in *syn*-treatment after 9 h with inhibition of 93%, 79% (*syn*) and 84%, 70% (*anti*) number of CFU.The decreasing biological availability of two isomers in soil extracted medium was also observed in Hoh et al.[3]It could be hypothesized that the very high hydrophobicity of DP leads to strong attachment to the soil particles, instead of biota.

For each of the two isomers,differentbehaviortowards the biota was observed. Tomyet al.[4]reported that the detectable frequency of the *syn*-form was more than that of *anti* in biota from Lake Winnipeg sediments. The *syn*-enrichment phenomena was caused by structural conformation of DP isomers in which *anti*-form showed less steric hinderance and hence was able to biologicallyattacktheC-skeleton of *anti*-structure as compared to the *syn*-form[3,4]. Moreover, trophic level could be an important biological attack tendency of DP isomers. In Tomy's study[4], he observed that *syn*-isomer was dominated in lower trophic level organisms such as zooplankton. It is quite correlated to our study in terms of a growth inhibition factor. Our bacteria were not able to degrade DP but they are the primary microorganism in the food web, so the potential interaction of *syn*-form to *E.coli*, G1 and PH-08 could be observed at high level compared to the *anti*-isomer. DP is a toxic chemical and hence the cell viability will be decreased majorly in highlybioaccumulated samples.

Intracellular ROS level of DP exposed bacteria

To estimate the induced stress from DP exposure to microorganism, we measured intracellular ROS level (Fig.2). The ROS intensity was observed significantly in PBS medium and it was relevant to the CFU

inhibition data. It can be seen that *syn*-isomer induced higher ROS level in *E.coli*(69% higher than *anti*-treatment) as well as PH-08 (49% higher than *anti*-inducer) in PBS medium while no significant difference in both isomers treatment was observed in G-1. Negligible interaction between DP isomers and microbiota in soil extracted medium could be observed (Fig. 2). The reason why low induced stress was observed in G1 sample is that G-1 is a plant promoting–growth which can support hormone for plant growth and induce some specific chemicals, such as siderophores, to reduce the attack of DP to bacteria. The high ROS level could imply that bacterial activities, especially G1 and PH-08 which play essential roles in environment, might be effected in DP treatment.

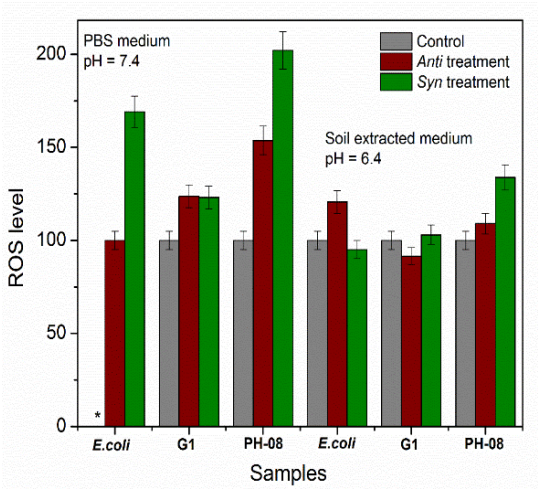

*Figure 2: The differences of ROS level were observed in E.coli, G1 and PH-08 after 2 h DP treatment. The exposed medium (PBS solution and soil extraction are in the left and right hand, respectively) could effected on risks of DP to bacteria. * means not detected*

Inhibited bioactivities of DP exposed bacteria

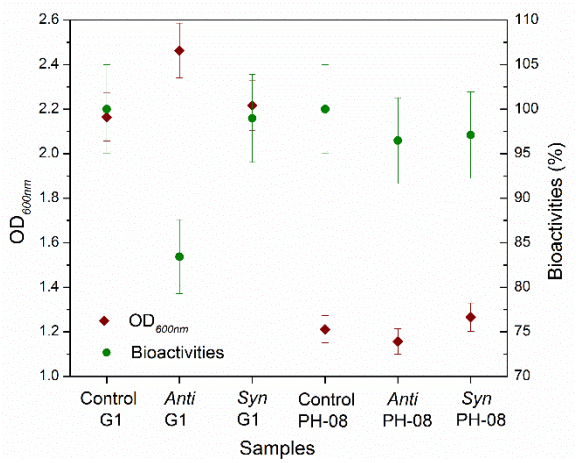

Figure 3: The effects of DP isomers on bioactivites of G1 and PH-08. The G1 (left) and PH-08 (right) were treated with 250 ppb of each isomer in their specific growth medium.

The effect of DP on bacterial activities, including phosphorus solubilizing and DF degrading capacities, was shown in Fig. 3. The insignificant differences between control and treated samples implied that DP isomers might not be a potential risk for inhibiting bioactivities. Only *anti*-treated sample of G1 showed 16.6% inhibition in the phosphorus solubilizing capacity. The comparative differences could not be observed in the other treatments. Moreover, in Fig.3 most of the samples, except*anti*-treated G1, showed the decrease trend

inbioactivtiesand was in accordance inhibition of growth measured via OD_{600nm} value. Our results were in agreement with Tomy's study[4]which concludedthatDP is a strong hydrophobic compound so it has a high potential to be attached on the particles' surface such as MSM medium components or insoluble phosphate $Ca_3(PO_4)_3$, instead of bacteria. This is the main reason which could explain the low bioactivity of DP in biota. However, in view of biodegradation studies, low or negligible interaction between DP and micro-biota in the suspended medium, especially soil system could prove to be a major disadvantage.

In conclusion, a certain risk of DP to micro-biota in aqueous and soil mimic condition was observed. The different behavior between *syn*- and *anti*-isomer was presented in cell viability, ROS level and bioactivties of *E.coli*, *Gluconacetobacterliquefaciens* sp. G1 and *Agrobacterium* sp. PH-08.In our study, we could determine that DP at low concentration could be toxic to bacteria but the toxicity level was decreased in suspended medium, such as soil extracted solution as well as specific growth medium. It was caused due to the high hydrophobicity of DP. This characteristic could limit the interaction between DP and microorganism, which could in turn be a limitation in terms of DP biodegradation.

Acknowledgements
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References
1.Sverko S,Tomy GT, Reiner EJ, LiYF,McCarryBEArnotJA,Law RJ, andHites RA. (2011); *Environ Sci Technol.*45(12):5088-98.

2. Betts KE. (2006);*Environ Sci Technol.*40(4):1090-1.

3.Hoh E, Zhu L, Hites RA. (2006); *Environ Sci Technol.* 40(4):1184-9.

4. Tomy GT, Pleskach K, Ismail N, Whittle DM, Helm PA, Sverko E, Zaruk D, Marvin CH. (2007); *Environ Sci Technol.* 41(7):2249-54.

5.Sverko E, Reiner EJ, Tomy GT, McCrindle R, Shen L, Arsenault G, Zaruk D, MacPherson KA, Marvin CH, Helm PA, McCarry BE. (2010); *Environ Sci Technol.* 44(2):574-9.

6. OxyChem. IUCLID Date Set; 07-NOV-2008.

7. Wu JP, Zhang Y, Luo XJ, Wang J, Chen SJ, Guan YT, Mai BX. (2010); *Environ Sci Technol*. 44(2):606-11.

8. Murphy J, Riley JP. (1962); *AnalyticaChimicaActa*. 27: 31-36