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SHORT COMMUNICATION

Activities and Toxicity of a Novel Plant Growth Regulator 2-Furan-2-yl-[1,3] Dioxolane

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Abstract The effect of activities of three substituted dioxolanes on wheat growth were assessed under laboratory/field conditions. Compound III, 2-furan-2-yl-[1,3]dioxolane, demonstrated good activity in shortening germination time, improving root growth, and increasing drought and salinity tolerance. In a water medium, the three substituted dioxolanes increased root numbers. Under water-deficit stress all treated seeds germinated by the fifth day, whereas controls did not germinate until after 6.5 days. These dioxolanes increased root number and root length. Compound III was the most outstanding of the three, with an increase of 227% in root number, 234% in root length, and 295% in shoot dry mass. Under salinity stress, these dioxolanes significantly improved growth (promotion of root number and shoot dry mass), although they had no effect on tissue moisture content. Field trials showed that compound III promoted root growth, increased root fresh weight significantly, and maintained a normal yield under water-deficit conditions. It showed low mammalian and environmental toxicity in various toxicologic tests. Results of *Salmonella typhimurium* reversion assay with and without a S9 mixture and the chromosomal aberration assay revealed that it had no mutagenic potential under experimental conditions.

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Introduction

Plant growth regulators (PGRs) are considered management tools in the producer's arsenal that can be used to ensure efficient production. Drought and salinity are two global problems that limit crop production. Synthetic PGRs have long been investigated for their ability to alter plant growth and development in an attempt to control growth and improve productivity (Nickell 1982; Davis and others 1988; Shang 2000). Various experiments have shown that some PGRs can improve crop resistance to different stresses, including water deficit. Benzyladenine (6-BA) is an active plant growth substance, and it can increase the drought resistance of different plants (Shang 2000). Triacontanol is a naturally occurring plant hormone that acts as a growth promoter. It has strong activity; it can stimulate the growth of crops at very low concentrations, and at high concentrations it can inhibit growth. It can increase root characteristics such as number of roots, total root length, maximum root length, and root yield and shoot characteristics such as the weight of leaves, petioles, and shoots (Bhattacharya and others 1996).

We were surprised to find that some substituted dioxolanes affect wheat growth. A lab assistant accidentally sprinkled the residue solutions of these compounds on some wilted wheat in flowerpots. Two days later, significant growth was found. We investigated further and found that they had good effect on growth in poor conditions and could be considered growth regulators (Figure 1).

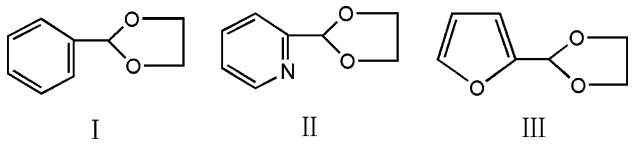

Fig. 1 The general structure of substituted dioxolanes: R = phenyl (compound I, 2-phenyl-[1,3]dioxolane); R = pyridyl (compound II, 2-pyridyl-[1,3]dioxolane); R = furan group (compound III, 2-furan-2-yl-[1,3]dioxolane)

There were few correlative reports about these substituted dioxolanes used in pesticides, and it did not appear that they had been investigated for plant-growth-regulating activity. Triazoles containing dioxolane are common fungicides, which have growth-regulating activity such as promotion of root growth and inhibition of stem elongation in seedlings (Bhattacharya and others 1996). Tkachenko and others (1974) performed studies on some radical isomerizations of 1,3-dioxacyclanes as feed additives. Most researchers emphasize the different catalyzers in the synthesis process (Meskens and Frans 1981; Yadav and others 2001; Ahmed and others 2002; Kazemi and others 2005), but little activity was found in these dioxolanes.

In this article the effects of these three dioxolanes on seed germination and root growth under drought stress and salinity stress in wheat are described. In addition, the toxicity of compound III is described to evaluate its environmental safety.

Materials and Methods

Synthesis and Synthetic Methods

Compounds were prepared according to the methods in the literature (Lipschutz and others 1985; Ott and others 1989; Brazili and others 2002) but the catalysts were improved. We used resin-D_{72}, a strongly acidic macroporous adsorption cation exchanger–sulfonic acid functionality resin, as a catalyst to get high yields under reflux and water separator conditions (Li and others 2006). Resin-D_{72} is a commercial domestic product that can be obtained easily and cheaply in China compared with Amberlyst-15 (U.S.). The reaction was simple, efficient, and did not involve any other additive.

Biological Tests

Solutions of compounds I–III were prepared in ethanol + distilled water (5 + 95 by volume) to a concentration of 50 mg L^{-1} and applied to seeds in a greenhouse. Triacontanol was used as a reference compound (positive control) at 2 mg L^{-1}. The concentration had been found to be the most effective in a preliminary experiment. The control was soaked in water. The test species was spring wheat (*Triticum aestivum* L). Seeds were provided by the Institute of Crop Germplasm Resources, Chinese Academy of Agricultural Science. Equal-sized and plump seeds were selected for the experiments. Seeds were soaked in the solutions for 12 h and germinated 5–10 days in a climatic chamber with natural lighting conditions at approximately 30°C/20°C day/night temperatures. The experiments were duplicated five times. Each experiment was made up of five treatments ordinal by control, triacontanol, compound I, compound II, and compound III. Each treatment was composed of 10 seeds. The number of germinant seeds was recorded and the effects on growth were determined by measuring number of roots, root length, and shoot dry mass. At harvest the plants were removed carefully and dried for 48 h at 70°C for determination of dry mass.

Seeds treated in a water medium

Seeds were soaked in water according to the method stated above and germinated for 5 days. During the experiments, seeds were watered every three days.

Seeds treated under water-deficit stress

According to the method of Chaves and others (2003), seeds were treated and germinated for 10 days with mannitol (osmotic potential = 0.6 MPa). Some experiments were conducted previously to establish the technique and to identify the appropriate mannitol level.

Seeds treated under salinity stress

The method used was that of Mass and Poss (1989) and Shazia (2001). Preliminary experiments were conducted to identify the appropriate NaCl treatment levels. In this study seeds were germinated for 10 days at 60 mM NaCl with electrical conductivity at 8.0 dS m^{-1} and a water potential of the salt solution of –0.31 MPa.

Field Trials

Field experiments were conducted in 2004 in an arid region (located in Pingyao city, Shanxi Province, in northwest China). The soil was sandy loam, the organic matter was 12.4 g kg^{-1}, and pH of the soil was 7.23. The field was used for rotation cropping with maize and wheat. The drought

treatment was applied by withholding irrigation throughout plant development after sowing. The controls were continuously maintained under optimal irrigation by irrigating regularly by hand. Test compound III was formulated as 200 g L^{-1} of emulsifiable concentrates (EC), and blank formulation was used as a reference. The control was soaked in water. Seeds were soaked in 50-mg L^{-1} solutions for 12 h and air-dried before planting. Each plot consisted of three rows 3 m long and 30 cm between rows. Plants within each row were 20 cm apart; to minimize border effects, adjacent plots were separated by 60 cm. The plants were grown under adequate nutrition and weeds and diseases were controlled. Foliage application was done twice separately at the elongation stage and at the heading period. The plot test was duplicated three times. Each treatment was applied to three plots for a total of 12 plots.

Four plots were selected at random to investigate seedling development at 7, 14, 21, and 28 days. Ten plants were harvested and measured at each time point in each plot for a total of 40 plants. Plant height and characteristics of ears of wheat like spike number, thousand kernel weight, and yield were evaluated at harvest. The yield was the kernel weight of 10 plants.

Toxicity Tests

Toxicology tests were performed according to the Organization for Economic Cooperation and Development (OECD) standard procedure. All study protocols were in compliance with Good Laboratory Practice (GLP) standards.

Acute Toxicity

Acute toxicity studies were performed on mice according to OECD guideline 2001. Specific pathogen-free male and female Wistar mice (initiated at 4.5 weeks old and treated at 5 weeks old, weight = 180–240 g) were used in the acute toxicity test offered by EACSMU, China. During the study animal rooms were under a 12-h light (150–300 lux):12-h dark cycle at a temperature of 22 ± 3°C, relative humidity 50 ± 10%, and 10–20 cycle/h of ventilation. Control and treated groups consisted of five males and five females. The animals were given a dose of 5 ml kg^{-1} of a solution of compound III in vegetable oil by gavage with a metal gastric cannula. It was tested at 215, 430, 860, 1720, and 2150 mg kg^{-1} body weight. The control group received 5 ml kg^{-1} of water. The animals were observed for overt signs of toxicity or death 0.5, 1, 2, and 4 h after dosing and subsequently once daily for 14 days following treatment. Body mass, water, and food consumption were measured over a 14-day period. After two week experimental period, the mice were killed.

In the histopathologic examination, tissue samples from the liver were preserved in 4% buffered formaldehyde. The tissues were embedded in paraffin wax and sections cut at 4 μm were stained with hematoxylin and eosin (H&E). Tissues from all animals were examined under a light microscope.

Salmonella typhimurium Reversion Assay (Ames test)

The *Salmonella typhimurium* reversion assay was performed according to the method of Ames revised edition (Maron and Ames 1983). The *Salmonella typhimurium* strains TA97, TA98, TA100, and TA102 described by Maron and Ames (1983) were provided by the Health Quarantine Station of Shanxi province (China). The S9 mixture was prepared from Chinese hamster liver as described in detail by Gomes-Carneiro and others (1998) to promote metabolic conversion of the test chemical. The solution was diluted with dimethylsulfoxide (DMSO) at 0.05, 0.22, 0.87, 3.48, and 12.2 mg ml^{-1}. The appropriate concentrations had been found in preliminary experiments (data not shown). Statistical significance of the differences among various groups was checked using the method of Kim and Margolin (1999).

Chromosomal Aberration Assay

Three groups of five male mice received by gavage a variable volume of compound III solution in olive oil at 18.7, 37.4, and 187.3mg kg^{-1} body weight once a day for 5 days. The negative control group received only olive oil. Mice in the positive control group were given a single injection of cyclophosphamide dissolved in water at a dosage of 40 mg kg^{-1} body weight. The chromosomal aberration test was performed and modified according to the method of Kim and others (2003). The animals were killed on the 13th day by cervical dislocation. The testis was dissected from each animal, seminiferous tubules were dissociated, and spermatogonium was collected in phosphate buffered solution. The cell concentration was calculated using a hemocytometer. Chromosomes were stained with diluted Giemsa (1:20) and evaluated by a single observer. A total of 100 well-spread metaphase cells (50 cells/tube) with 40 ± 2 chromosomes per animal were scored for gaps, breaks, and exchanges. Chromosome aberrations were scored, and the total percentage of abnormal cells was expressed for statistical analysis to observe significant differences in various types of chromosome aberrations between the treated and the control groups.

Statistical Analysis

All statistical analyses were performed using SPSS ver. 11.0 for Windows. The results were presented using both the mean value [mean ± standard deviation (SD)] and percent data, analyzed by a one/two-way analysis of variance (ANOVA). When significant according to the ANOVA, the comparisons between treatments were made using Duncan's multiple-range test for mean significances ($p = 0.05$); the levels of significance were established at $p < 0.05$ and $p < 0.01$.

Results

Substituted Dioxolanes on Root Growth

The results of various experiments showed that these substituted dioxolanes had some positive effect on root growth. In the water medium, all seeds germinated and grew well. All substituted dioxolanes improved root numbers, but triacontanol did not (Figure 2). Most chemicals promoted shoot dry mass and compound III increased it up to 246% ($p < 0.01$), but compound I did not. Most treatments had no effect on root length, whereas compound II restrained it. Under water-deficit stress, all treated seeds germinated on the fifth day, whereas the control did not until 6.5 days. This showed that treatments could promote seed germination in mannitol solution. All treatments improved root numbers under water deficit (Figure 3). Triacontanol, compound II, and compound III improved root lengths, whereas compound I did not. Only compounds II and III increased shoot dry mass. Compound III was the most outstanding of all with an increase of 227% in root number, 234% in root length, and 295% in shoot dry mass. Triacontanol had some stimulative effect on the root system under water deficit but the effect was less than compound III. Under salinity stress, all seeds germinated, although the treatments germinated 1 or 2 days before the control. All substituted dioxolanes significantly improved growth, although they had no effect on tissue moisture content (Figure 4). Triacontanol had no distinct effect in this test.

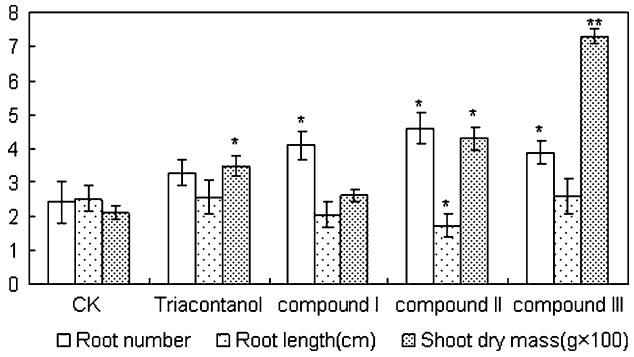

Fig. 2 Effects of compounds I, II, and III at 50 mg L^{-1} on root growth (in a water medium). The concentration of triacontanol was 2 mg L^{-1} and the control was soaked in water. Plants were harvested on the fifth day. The control was at the two-leaf stage, those treated with triacontanol and compound I were at the two- to three-leaf stage, and the others were at the three-leaf stage. The *y* axis was just a quantity for root number, no unit; root length was in centimeters; shoot dry mass was grams × 100 to make the number in order. The data represent the mean of five measurements. Vertical bars represent one standard deviation of the mean. Significant when compared with the respective control group: $*p < 0.05$ and $**p < 0.01$

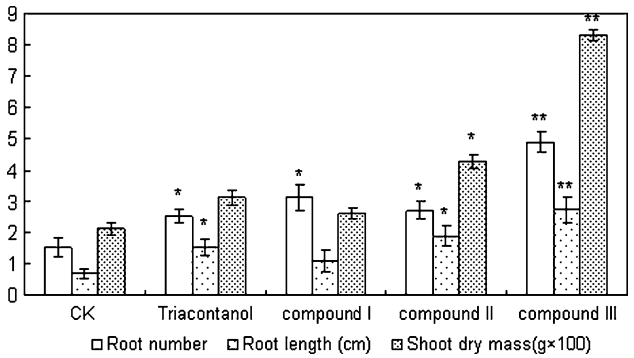

Fig. 3 Effects of compounds I, II, and III at 50 mg L^{-1} on root growth (under water-deficit stress). The concentration of triacontanol was 2 mg L^{-1} and the control was soaked in water. Plants were harvested on the tenth day. The control was at the two-leaf stage, and the treated were at the two- to three-leaf stage. The *y* axis was just a quantity for root number, no unit; root length was in centimeters; shoot dry mass was grams × 100 to make the number in order. The data represent the mean of five measurements. Vertical bars represent one standard deviation of the mean. Significant when compared with the respective control group: $*p < 0.05$ and $**p < 0.01$

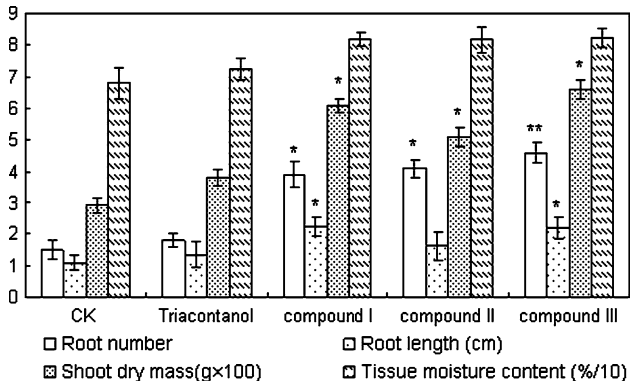

Fig. 4 Effects of compounds I, II, and III at 50 mg L^{-1} on root growth (under salinity stress). The concentration of triacontanol was 2 mg L^{-1} and the control was soaked in water. Plants were harvested on the tenth day. The control was at the two-leaf stage and the treated were at the two- to three-leaf stage. The *y* axis was just a quantity for root number, no unit; root length was in centimeters; shoot dry mass was grams × 100; and tissue moisture content was %/10 to make the number in order. The data represent the mean of five measurements. Vertical bars represent one standard deviation of the mean. Significant when compared with the respective control group: $*p < 0.05$ and $**p < 0.01$

Compound III on Seedling Development and Yield

Compound III was selected for further studies because it showed the best results in the laboratory experiments. Blank formulation was applied to eliminate the formulants effect. Field trials showed its effect on seedling development. Root fresh weight was increased in seedlings at 7 days (43%) and 14 days (33%) after treatment (Figure 5), but there was no effect on plant height at any time after treatment (data not shown). The check experiments showed that blank formulation had no effect on plant growth. The effect of compound III decreased gradually, so another application to the plants was needed. At harvest, the plants treated with the blank formulation showed poor growth, resulting in significant yield loss, but those treated with compound III grew as well as the control and maintained yields under water deficit. Compound III promoted root growth, increased root fresh weight significantly (Figure 4), and maintained a normal yield (Figure 6); this agreed with the previous experiments. Similarly, plant height was not influenced by drought. From these results, the application of compound III, 2-furan–2-yl-[1,3]dioxolane, was effective for plant growth and yield under water deficit conditions in field trials.

Toxicity

Acute Toxicity

No clinical signs of toxicity were noted and no deaths occurred in animals treated with 215 and 430 mg kg^{-1} body weight. The animals in the high-dose group (2150 mg kg^{-1}) showed clear clinical signs of toxicity, including restlessness, aggressiveness, and green excretion. All the rats at the high-dose level died after 1–3 days of dosing, within 1 h after dosing, or were sacrificed due to poor general condition.

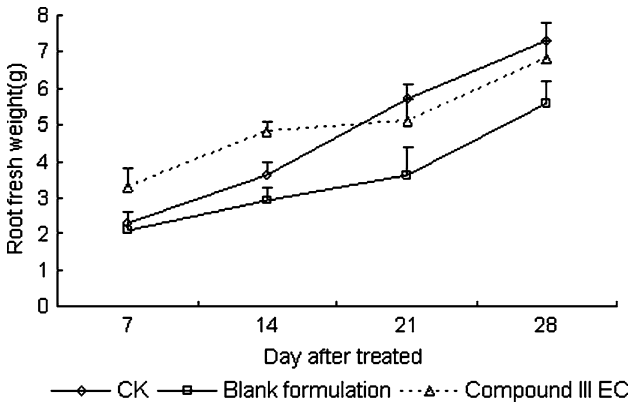

Fig. 5 Effect of compound III EC at 50 mg L^{-1} on root development. The data were investigated 7, 14, 21, and 28 days after treatment. Individual data points represent the mean of four measurements. Each measurement was made on 10 plants in the plot. Vertical bars represent one standard deviation of the mean. Significant when compared with blank formulation and control group: $*p < 0.05$

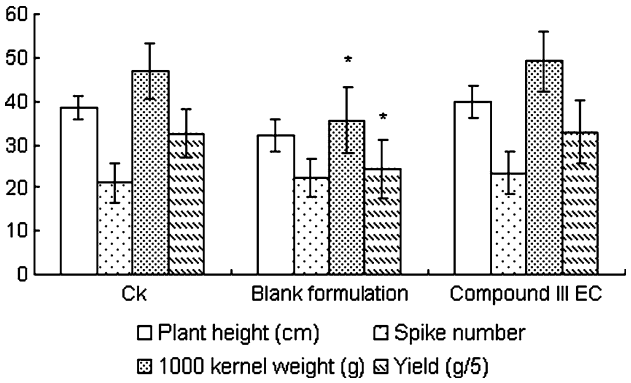

Fig. 6 Effect of compound III EC at 50 mg L^{-1} on yield under water deficit. Plant height, characteristics, and yield were investigated at harvest. The *y* axis was just a quantity for plant height in centimeters; spike number was grain; 1000 kernel weight was in grams; and yield was grams/5 to make the number in order. Individual data points represent the mean of four measurements. Each measurement was made of 10 plants in the plot. Vertical bars represent one standard deviation of the mean. Significant when compared with the respective control group: $*p < 0.05$

In the studies the rats were subjected to necropsy. Because all the deaths were dose-related, with similar clinical symptoms, and no other reasons for death were found at necropsy, the deaths were considered to be related to the treatment.

Body weight gain was adversely affected in all treatment groups in a dose-related manner. Statistically significant differences from their controls were detected in males and females receiving 1720 and 2150 mg kg^{-1} of compound III throughout most of the study period. Water and food consumption were depressed in a dose-related manner. Statistically significant differences from the controls were demonstrated in rats of both sexes receiving 1720 and 2150 mg kg^{-1} of compound III.

Histologic examination of liver tissue from the low-dose (215 kg^{-1}) animals did not reveal any signs of hepatotoxicity. In the rats treated with the high dose of compound III (2150 mg kg^{-1}), necrotic foci or single-cell necrosis were seen in two of five animals. All other liver findings were considered to be part of the normal background data of this strain of rats and were considered to be of no toxicologic significance.

Mutagenicity

Ames tests using *Salmonella typhimurium* TA97, TA98, TA100, and TA102 were performed with and without a metabolic activation system (S9 mixture). No significant

increases in revertants were observed in either test, and the activity of the S9 fraction was found to be satisfactory. The number of revertant colonies in the assay was less than twice the number of spontaneous revertants (MI ≤ 2). However, the positive control showed a significant increase in the number of mutation colonies compared with the negative control. These results strongly suggest that compound III did not have any mutagenic potential on *Salmonella* strains. No statistically significant increases in aberrations were observed for all concentrations tested, except that the positive control reached 9.6% frequency of cells with chromosomal aberration. More toxicologic data such as subchronic toxicity, chronic toxicity, and carcinogenic effects need to be collected in further research.

Discussion

In the field plants are subjected to a more gradual stress because water availability in the soil does not change abruptly and, therefore, plant responses might be different than those under lab conditions. Slower growth had been suggested as an adaptive feature for plant survival under stress (Zhu 2002). Under water-deficit stress, the control showed restrained root growth (Figure 3). This was very similar to the root growth decrease under water stress (Zhu 2002; Chaves and others 2003; Flexas and others 2004). Shukla and others (1992) verified that triacontanol produced a statistically significant positive effect on plant height and leaf and herbage yields. In our experiments triacontanol significantly affected shoot dry mass in water medium (Figure 2). This was consistent with the literature. It is generally accepted that both the quantity of roots and tissue moisture content of seedlings or plants are the most important characteristics in drought stress (Chaves and others 2003). In this study all chemicals promoted the root system (Figure 3) but did not act on the tissue moisture content.

Salinity impaired seed germination, reduced nodule formation, retarded plant development, and reduced crop yield (Greenway 1980). The root system is the first organ to be injured under salinity stress, so the better the root growth, the better the plant development and the higher the yield. When the leaves hold more tissue moisture, the plant can receive and hold more water, and normal physiologic functions can continue longer, thus lowering the salt damage (Maas and Poss 1989). In this study the root growth of the control under salinity stress was inhibited compared to that in the water medium (Figures 2, 4). This was similar to other studies. All chemicals but triacontanol showed endurance under salinity stress by improving the root system, and compound III notably increased root number (Figure 4). These substituted dioxolanes had significant effects on root growth, whereas they had no effect on tissue moisture content. This was very dissimilar to the results of Maas and Poss (1989). From our results, compound III, namely, 2-furan-2-yl-[1,3]dioxolane, was the optimal one in the simulation test of adversity.

Chemical PGRs have been widely used to adjust plant growth and improve yield under various environments (for example, soil and rainfall). Water-deficit stress, which could arise from many environmental conditions, including drought, salinity, or extremes in temperature, induces numerous biochemical and physiologic responses in plants (Hanson 1982). Under water-deficit conditions, plant growth and yield are substantially reduced. In our field trials, the blank formulation decreased root fresh weight and induced significant yield loss under water-deficit conditions (Figure 5), whereas treatment with compound III improved root growth and maintained yield (Figure 6). All treatments had no effect on plant height in the seedling period and harvest. Ongoing field tests continue to evaluate its application and effect. The recommended rate of use for this chemical is 12–24 g AI ha^{-1} (data not shown) to promote plant growth and withstand poor growing conditions.

The acute toxicity (LD_{50}) of compound III to mice was about 562 mg kg^{-1}, with no differences between the sexes. Death was dose-related. Histopathologic examination of liver tissue from the low-dose application did not reveal any signs of hepatotoxicity. However, necrotic foci or single-cell necrosis was seen in two of five animals after high-dose treatment. Mutagenic experiments showed that it was negative in both the mutation and chromosome aberration tests and they revealed that it had no mutagenic potential under experimental conditions. These results indicated that compound III had a low toxic effect on mice. This was enough toxicologic data to gain temporary pesticide registration in China.

In brief, compound III, 2-furan-2-yl-[1,3]dioxolane, is suited for plant treatment in poor crop conditions, especially in the seedling stage. With low environmental and mammalian toxicity, we hope it can soon be developed further and commercialized as an original plant growth regulator in China. Further research is needed to study the effect on adversity stress. In addition, the residue, mechanism, and environmental assessment of 2-furan-2-yl-[1,3]dioxolane await further research.

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