Provisional translation

The Food Safety Commission

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Interpretation of the liver hypertrophy in the toxicological evaluation of pesticides (agricultural chemicals)

The liver hypertrophy commonly observed in toxicological studies of a pesticide is indicative of an adaptive response of the organism or an adverse effect of the pesticide. FSCJ (the expert committee on pesticides) revised in this document the current approach to the liver hypertrophy for enabling every expert committee to consistently interpret whether the liver hypertrophy observed in a toxicological study is indicative of a biological adaptation or an adverse effect of pesticide.
Since this is a summary based on the present scientific knowledge, the approach would be revised as necessary after reviewing their provisions taking into account international trends of the toxicological evaluation procedures and new scientific knowledge within and outside Japan.

1. Background

Although increases in the organ weight of the liver and/or hepatocellular hypertrophy are the most popular effects of exposure of animals to chemical substances in the toxicity studies, it has long been debated if these changes are the steps which can lead to hepatic disorder or tumors. On the other hand, the liver is an organ that metabolizes chemical substances, and it is known that the metabolic activity induces various drug-metabolizing enzymes in organelles of hepatocytes and thus results in hyperplasia of the organelles which consequently causes changes in the organ weight or hepatocellular hypertrophy.
Accordingly, a question whether these changes are adverse effects on the living organisms such as humans or results of adaptations has been a subject of many worldwide discussions. Recently, a general consensus regarding this question is being internationally accepted as follows; a hepatocellular hypertrophy caused by an exposure to a chemical substance is an adaptive change but not an adverse effect as far as the homeostasis of the living organism is maintained. On the other hand, when the exposure results in a hepatocellular hypertrophy accompanied with a hepatic dysfunction such as a hepatocellular injury detected by a histopathological examination or an abnormal value related to a hepatic toxicity determined by a biochemical examination of blood, the

hepatocellular hypertrophy should be judged as the toxic effects since the homeostasis in the relevant organism is being lost.
The expert committee on pesticides of FSCJ has conducted the toxicological evaluation of various pesticides on the basis of a consistent standard where the change is judged as a toxic effect when a concomitant increase in the absolute and relative organ weight of the liver is observed with a significant difference and when hepatocellular hypertrophy is observed. However, such an approach may lead a toxicological evaluation of the hepatocellular hypertrophy to a misjudgment where a physiological response of living organism is mistaken for a toxic effect. Thus, FSCJ considered it necessary to examine whether the hepatocellular hypertrophy is appropriate as an end point in a risk assessment.

2. Definition of The liver hypertrophy

In this document, the liver hypertrophy is defined as a morphological enlargement of hepatocytes (hepatocellular hypertrophy), resulting in an increase in the organ weight, which is caused by enhanced hepatocellular function in response to an extrinsic factor.

(1) Morphological changes
"Hepatocellular hypertrophy" is the enlargement of individual hepatocytes at microscopic level. Cytoplasm of the enlarged hepatocytes, in comparison to the cytoplasm of the normal cells, is often observed as ground-glass like or fine granular eosinophilic cytoplasm. An electron microscopic morphology characterizes the cell enlargement due to a proliferation of smooth endoplasmic reticulum (sER) or microbody in the hepatic cytoplasm.
In the case of hepatocellular hypertrophy accompanied by the induction of drug-metabolism related proteins, hypertrophy often starts to be observed in the centrilobular hepatocytes, spreading to the intermediate zone as it progresses, and finally observed as diffuse hypertrophy all around the lobule of the liver. When liver hypertrophy is caused by peroxisome proliferation、the hepatocellular hypertrophy often presents as a diffuse change affecting all zones of the liver lobule.
Hepatocellular hypertrophy is distinguished from enlargement of hepatocytes by hydropic degeneration, accumulation of lipids or glycogen in hepatocytes, and hyperplasia of mitochondria.

(2) Functional changes
Hepatocellular hypertrophy is considered as a functional adaptation of the organisms for maintaining the homeostasis toward xenobiotic substances that are taken up in the living body, inducing hepatocellular proteins involved in hepatocellular drug metabolisms such as drug-metabolizing enzymes and transporters, thus enhancing the rate of metabolism and excretion of the xenobiotics. Details of the functional adaptation are described in the reference at the end of this document.

3. Fundamental stance on the interpretation of the liver hypertrophy in a toxicological evaluation

1) Principle

Because hepatocellular hypertrophy is the enhancement of the hepatic function as a response of the living organisms toward xenobiotic factors for maintaining the homeostasis, the liver hypertrophy is regarded as an adaptive change but not a toxic effect as long as the homeostasis is maintained. On the other hand, a the liver hypertrophy that results in a failure of homeostasis by responding to the level above the limit of the homeostatic capacity of the organism is regarded as an adverse effect on the living organism but not an adaptation.

The liver hypertrophy induced by a xenobiotic needs to be distinguished whether it is a toxic effect or an adaptive change based on comprehensive evaluation of the effects observed with relevant substance.

2) Detailed Exposition

(1) Changes to be recognized as toxic effects

① Types of hepatocellular hypertrophy

It should be considered that the hepatocellular hypertrophy relevant to the following cases have risks of being toxic effects of a xenobiotic.

- When periportal hepatocellular hypertrophy is observed by a histopathological examination.
- When balloon like large hepatocytes or hydropic degenerations, but not ground-glass like or fine granular cytoplasm are observed microscopically.
- When the electron-microscopic examination reveals that the hepatocellular hypertrophy that are with changes of the intracellular organelles other than the increases in the number of sER or hyperplasia of microbody.

Since hepatocellular hypertrophy of different types are usually distinguished by a histopathological examination, the regions of the hypertrophy or morphological characteristics of the hepatocytes are important information for distinction of the liver hypertrophy.

However, even if the regions of the hypertrophy are not described, whether the hypertrophy is a toxic effect or not should be judged comprehensively considering other information such as the degree of changes.

② When degeneration or necrosis of hepatocytes (including monocellular necrosis) is recognized and changes in the indexes of relevant inflammatory responses for such degeneration or necrosis are detected

A failure of homeostatic capacity of the liver by functionally exceeding the limit of the capacity results into hepatocellular injury followed by the cell death. These results are morphologically observed as degeneration or necrosis (including monocellular necrosis) of hepatocytes and relevant inflammatory responses. In addition, fibrosis or the formation of granulomas are occasionally observed after inflammation become chronic.
As for blood biochemical indexes, an increase in the activity of alanine aminotransferase (ALT), a deviation enzyme from hepatocytes due to the functional break down, is the most sensitive index. An increase in the activity of aspartate aminotransferase (AST), another deviation enzyme likewise, and an increase in alkaline phosphatase (ALP) which is a hepatocellular microsomal enzyme are considered as potential blood biochemical indexes of hepatocellular injury. Note, however, that changes in the activity of other isozymes originated from other organs, not only from the liver, affect ALP activity. A change in ALP activity, particularly in dogs, should be carefully judged if it reflects the hepatotoxicity or not, because dogs used in the toxicity study are generally in their growth period (4 to 6 month old at the starting of the study) that is the same period where bone-derived ALP changes.

③ When changes of the biliary system accompany the liver hypertrophy
The liver hypertrophy occasionally is accompanied with histopathological findings that indicate changes in biliary system. These changes are detected as degeneration / necrosis of the biliary system or bile duct, inflammatory response and hyperplasia of bile duct histopathologically, and detected as increases in blood biochemical indexes such as bilirubin or γ-glutamyltranspeptidase (GGT).
Although direct association between these changes and hepatocellular hypertrophy is difficult to be revealed by ordinal toxicity evaluation studies, the potential risk that the liver hypertrophy is a toxic effect should be considered when the liver hypertrophy is observed to be accompanied with these changes.

④ When changes of lipid metabolism accompany the liver hypertrophy
Histopathological findings that indicate lipid accumulation in hepatocytes are occasionally observed with the liver hypertrophy. Changes leading to such lipid accumulation in hepatocytes are reflected in blood biochemical indexes related to lipid metabolism such as increased triglyceride level or increased cholesterol level. In addition, lipid accumulation in hepatocytes are sometimes observed with degeneration / necrosis or inflammatory response of hepatocytes.
Although direct association between hepatocellular hypertrophy and lipid metabolism disorder is difficult to be revealed by ordinary toxicity evaluation studies in many cases, the potential risk that the liver hypertrophy is a toxic effect should be considered when the liver hypertrophy is observed to be accompanied with relevant changes since these indexes are considered to vary upon changes in hepatocellular lipid metabolism.

⑤ When pigmentations accompany the liver hypertrophy

The liver hypertrophy is occasionally accompanied with certain histopathological findings such as cytoplasmic brown pigmentation in monocytic phagocytes including liver cells and Kupffer cells. Pigmentation is induced by different factors depending on the type of pigment. In many cases, porphyrin deposition suggests changes of hem metabolism in hepatocytes, lipofuscin deposition suggests aging or enhanced hepatocellular lipid peroxidation, and increased hemosiderin deposition suggests biochemical response to hemolysis occurred in the living organism.

Although direct association between hepatocellular hypertrophy and pigmentation is difficult to be revealed by ordinary toxicity evaluation studies in many cases, the potential risk that the liver hypertrophy is a toxic effect should be considered when the liver hypertrophy is observed to be accompanied with relevant changes since increases in pigmentation are considered to be often induced by toxic changes in the liver or in the whole organism.

(2) The liver hypertrophy to be recognized as an adaptive response

When changes in the indexes related to hepatic disorders, as was described in (1), are not observed with a hepatocellular hypertrophy, the observed liver hypertrophy shall be interpreted as an adaptive effect but not an adverse effect, because homeostasis is considered to have been maintained in the organism.

On the other hand, when nuclear receptors and nuclear transcription factors are thought to be involved and their activation is observed, the finding is possibly an evidence for an adaptive response. Therefore, a temporal search for multiple enzymes (molecular species) in toxicological studies is useful to understand the characteristics of protein induction by the relevant chemical substance. However, the results should be used strictly for revealing the mechanisms and are not essential requirements for a toxicity evaluation. Hence, it should be noted that whether the observation of hepatocellular hypertrophy is indicative of an adaptive or an adverse event can’t be determined based solely on the results of such a temporal search.

3) Factors to consider

(1) When the liver changes caused by a low dose in the same toxicological study where a high dose of the chemical caused a hepatic disorder.

The liver hypertrophy can be induced by a low dose of a chemical in the same study where a highdose of the chemical caused hepatic disorders. If the liver hypertrophy by the low dose is not accompanied with histopathological changes such as degeneration/necrosis of hepatocytes or inflammatory responses or with changes in blood biochemical indexes of hepatotoxicity, such hypertrophy shall be interpreted as an adaptive response to maintain homeostasis in the whole organism.

Details of the histopathological findings and comprehensive analysis of the blood biochemical data are essential for such an interpretation.

(2) When the liver hypertrophy is a transient change.
The liver hypertrophy that is observed in short-term studies sometimes is not observed in long-term studies even at the same doses. In such cases, the liver hypertrophy is indicative of the adaptive response.

(3) When weight of the liver is increased.
An increase in the weight of the liver is an important indicator for detecting any change in the liver.
The increase in the weight of the liver is distinguished as the increase when the relative weight is increased with a statistically significant difference in the case of rodents. In the case of non-rodents, changes in the weight of the liver may not be distinguishable only on the basis of changes in the relative weight because of large individual differences in the organ weight of non-rodents. Therefore, the increase in the weight of the liver is distinguished as the increase when both the relative and absolute weight significantly increased in the case of non-rodents.
It should be considered whether the increase in the weight of the liver is an adverse effect based on the standards described in (1) of 2). An increase in the weight of the liver and /or hepatocellular hypertrophy, accompanied with none of adverse changes listed in (1) of 2), is considered as an adaptive response. Therefore, a hepatic toxicity shall not be concluded simply based on an increase in the weight of the liver and /or hepatocellular hypertrophy. In addition, hepatocellular hypertrophy might be the secondary response to the suppressed body weight if an increase in the relative weight of the liver without histopathlogical finding of hepatocellular hypertrophy is observed only at the high dose exposure. Therefore, clear evidences are required for interpreting an increase in the relative weight of the liver as a toxic event.

(4) Data of blood biochemical examinations
In blood biochemical examinations in toxicity studies, many indexes are determined including the changes that occur with the liver hypertrophy. Changes in the value of each index is interpreted as a toxic effect basically if the difference is statistically significant.
However, hepatic toxicity from each index may be interpreted by expert judges considering the normal range at the relevant age in weeks or background data of the test conducting institutes.

(5) When changes in the function of the thyroid gland accompany.
Induction of the second phase drug-metabolizing enzymes in the liver may enhance the metabolism of thyroid hormone resulting in the secondary increase of thyroid-stimulating hormone and hypertrophy / hyperplasia of follicular epithelial cell of the thyroid gland. When such changes in the thyroid gland are observed, hepatic capacity of homeostasis likely exceeded the normal range and thus resulted in disorder of homeostasis not only in the liver but also in the whole body such as abnormal function of the pituitary and thyroid gland.

Therefore, if changes in the function of the thyroid gland are observed, whether the liver hypertrophy observed in relevant study is the toxic response should be carefully interpreted.

Note:

Changes in foreign matter-responsive nuclear receptors and nuclear transcription factors.

Enhanced biosynthesis of metabolism related proteins has been reported to be induced through activation of foreign matter-responsive nuclear receptors and nuclear transcription factors as the major adaptive response of the liver.

The known foreign matter-responsive nuclear receptors include constitutive androstane/active receptor (CAR), pregnane X receptor (PXR), and peroxisome proliferator-activated receptor alpha (PPARα), and the nuclear transcription factors include aryl hydrocarbon receptor (AhR) and NF-E2=related factor 2 (Nrf2).

Induction of the proteins related to the metabolism of foreign matters is by itself a reversible adaptive response of the organism to changes in the external circumstances for maintaining homeostasis, and it is often observed prior to the hepatocellular hypertrophy as changes of intracellular organelles.

Besides, there are the nuclear receptors, particularly such as CAR, PXR and PPARα, that are highly associated with lipid metabolism. These nuclear receptors often have association with the changes in the parameters related to lipid in blood.

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