

WHAT IS CLAIMED IS:

1. An *in vitro* method of screening for renal proximal tubular toxicity of a compound, the method comprising:

contacting a test compound that is to be screened for renal proximal tubular cell toxicity with a test population of renal proximal tubular cells grown attached to a support; and

measuring one or both of the following features of the renal proximal tubular cells in the test population: cell area, and area of nucleus: area of whole cell ratio;

comparing the one or more features of the test population with the corresponding one or more features of a control population that has not been contacted with the test compound; and

identifying the test compound as toxic for renal proximal tubular cells based on one or both of a decrease in cell area, and an increase in area of nucleus: area of whole cell ratio of the test population relative to the control population.

2. The method of claim 1, wherein the measuring comprises use of automated image analysis techniques.

3. The method of claim 1 or 2, further comprising:

examining arrangement of tubulin; and

comparing the arrangement of tubulin of the test population with arrangement of tubulin of a control population that has not been contacted with the test compound;

wherein the identifying of the test compound as toxic for renal proximal tubular cells is further based on a change in the arrangement of tubulin of the test population relative to the control population.

4. The method of any one of claims 1 to 3, further comprising:

determining cell numbers of the renal proximal tubular cells in the test population; and

comparing cell count of the test population with cell count of a control population that has not been contacted with the test compound;

wherein the identifying of the test compound as toxic for renal proximal tubular cells is further based on a decrease in cell numbers of the test population relative to the control population.

5. The method of any one of claims 1 to 4, further comprising:

examining arrangement of F-actin; and

comparing the arrangement of F-actin of the test population with arrangement of F-actin of a control population that has not been contacted with the test compound;

wherein the identifying of the test compound as toxic for renal proximal tubular cells is further based on a change in the arrangement of F-actin of the test population relative to the control population.

6. The method of any one of claims 1 to 5, further comprising:

examining cell roundness; and

comparing the cell roundness of the test population with cell roundness of a control population that has not been contacted with the test compound;

wherein the identifying of the test compound as toxic for renal proximal tubular cells is further based on an increase in rounding of the proximal tubular cells of the test population relative to the control population.

7. The method of any one of claims 1 to 6, further comprising prior to said examining and/or said determining, labeling the test population with a detectable label.

8. The method of claim 7, further comprising labeling the control population with the detectable label.

9. The method of claim 7 or claim 8, wherein the detectable label is a fluorescent label, or a coloured label.

10. The method of claim 7 or claim 8, wherein the detectable label is a nuclear label, a whole cell label, a cytoplasmic label, a label for cytoskeletal proteins or a cell surface label.

11. The method of any one of claims 1 to 10, wherein the renal proximal tubular cells are derived from somatic cells or from stem cells.

12. The method of claim 11, wherein the renal proximal tubular cells are derived from somatic cells and are primary cells or are cells from a stable cell line.

13. The method of claim 12, wherein the renal proximal tubular cells are human primary renal proximal tubular cells, HK-2 cells, or LLC-PK1 cells.
14. The method of claim 11, wherein the renal proximal tubular cells are derived from stem cells and are differentiated from embryonic stem cells, mesenchymal stem cells, or induced pluripotent stem cells.
15. The method of any one of claims 1 to 14, wherein the renal proximal tubular cells are human renal proximal tubular cells.
16. The method of any one of claims 1 to 14, wherein the renal proximal tubular cells are non-human renal proximal tubular cells.
17. The method of any one of claims 1 to 16, wherein said contacting is performed over a period of time of about 8 hours or longer.
18. The method of any one of claims 1 to 17, wherein said contacting is repeated one or more times in a period of from about 3 to about 14 days.
19. The method of any one of claims 1 to 18, wherein said contacting comprises adding the test compound to the test population of renal proximal tubular cells at a concentration of about 0.001 to about 1000 $\mu\text{g/ml}$.

WHAT IS CLAIMED IS:

1. An *in vitro* method of screening for renal proximal tubular toxicity of a compound, the method comprising:

contacting a test compound that is to be screened for renal proximal tubular cell toxicity with a test population of renal proximal tubular cells grown attached to a support; and

examining-measuring one or more-both of the following features of the renal proximal tubular cells in the test population: cell area, and area of nucleus: area of whole cell ratio, and arrangement of tubulin; and

comparing the one or more features of the test population with the corresponding one or more features of a control population that has not been contacted with the test compound; and

wherein-identifying the test compound as toxic for renal proximal tubular cells based on one or more-both of a decrease in cell area, and an increase in area of nucleus: area of whole cell ratio, and a change in the arrangement of tubulin of the test population relative to the control population-is indicative that the test compound is toxic for renal proximal tubular cells.

2. The method of claim 1, wherein the measuring comprises use of automated image analysis techniques.

3. The method of claim 1 or 2, further comprising:

examining arrangement of tubulin; and

comparing the arrangement of tubulin of the test population with arrangement of tubulin of a control population that has not been contacted with the test compound;

wherein the identifying of the test compound as toxic for renal proximal tubular cells is further based on a change in the arrangement of tubulin of the test population relative to the control population.

- 2.4. The method of any one of claims 1 to 3, further comprising:

determining cell numbers of the renal proximal tubular cells in the test population; and

comparing cell count of the test population with cell count of a control population that has not been contacted with the test compound;

~~wherein the identifying of the test compound as toxic for renal proximal tubular cells is further based on a decrease in cell numbers of the test population relative to the control population is indicative that the test compound is toxic for renal proximal tubular cells.~~

~~3.5.~~ The method of ~~any one of claims 1 or 2~~ to 4, further comprising:

examining arrangement of F-actin; and

comparing the arrangement of F-actin of the test population with arrangement of F-actin of a control population that has not been contacted with the test compound;

~~wherein the identifying of the test compound as toxic for renal proximal tubular cells is further based on a change in the arrangement of F-actin of the test population relative to the control population is indicative that the test compound is toxic for renal proximal tubular cells.~~

~~4.6.~~ The method of any one of claims 1 to ~~3~~5, further comprising:

examining cell roundness; and

comparing the cell roundness of the test population with cell roundness of a control population that has not been contacted with the test compound;

~~wherein the identifying of the test compound as toxic for renal proximal tubular cells is further based on an increase in rounding of the proximal tubular cells of the test population relative to the control population is indicative that the test compound is toxic for renal proximal tubular cells.~~

~~5.7.~~ The method of any one of claims 1 to ~~4~~6, further comprising prior to said examining and/or said determining, labeling the test population with a detectable label.

~~6.8.~~ The method of claim ~~5~~7, further comprising labeling the control population with the detectable label.

~~7.9.~~ The method of claim ~~5-7~~ or claim ~~6~~8, wherein the detectable label is a fluorescent label, or a coloured label.

~~8.10.~~ The method of claim ~~5-7~~ or claim ~~6~~8, wherein the detectable label is a nuclear label, a whole cell label, a cytoplasmic label, a label for cytoskeletal proteins or a cell surface label.

~~9~~11. The method of any one of claims 1 to ~~8~~10, wherein the renal proximal tubular cells are derived from somatic cells or from stem cells.

~~10~~12. The method of claim ~~9~~11, wherein the renal proximal tubular cells are derived from somatic cells and are primary cells or are cells from a stable cell line.

~~11~~13. The method of claim ~~10~~12, wherein the renal proximal tubular cells are human primary renal proximal tubular cells, HK-2 cells, or LLC-PK1 cells.

~~12~~14. The method of claim ~~9~~11, wherein the renal proximal tubular cells are derived from stem cells and are differentiated from embryonic stem cells, mesenchymal stem cells, or induced pluripotent stem cells.

~~13~~15. The method of any one of claims 1 to ~~12~~14, wherein the renal proximal tubular cells are human renal proximal tubular cells.

~~14~~16. The method of any one of claims 1 to ~~12~~14, wherein the renal proximal tubular cells are non-human renal proximal tubular cells.

~~15~~17. The method of any one of claims 1 to ~~14~~16, wherein said contacting is performed over a period of time of about 8 hours or longer.

~~16~~18. The method of any one of claims 1 to ~~15~~17, wherein said contacting is repeated one or more times in a period of from about 3 to about 14 days.

~~17~~19. The method of any one of claims 1 to ~~16~~18, wherein said contacting comprises adding the test compound to the test population of renal proximal tubular cells at a concentration of about 0.001 to about 1000 µg/ml.