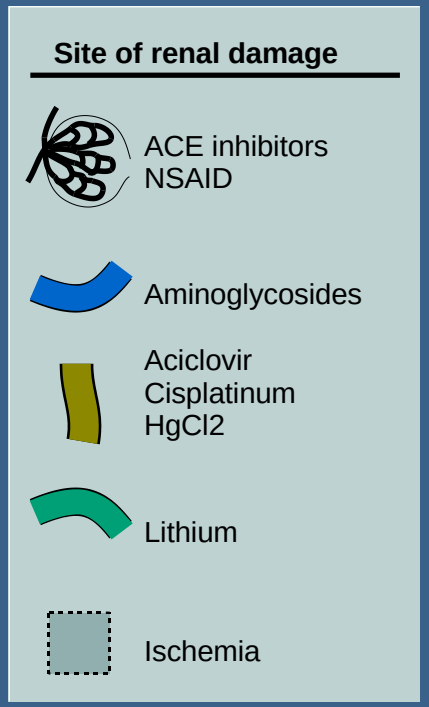
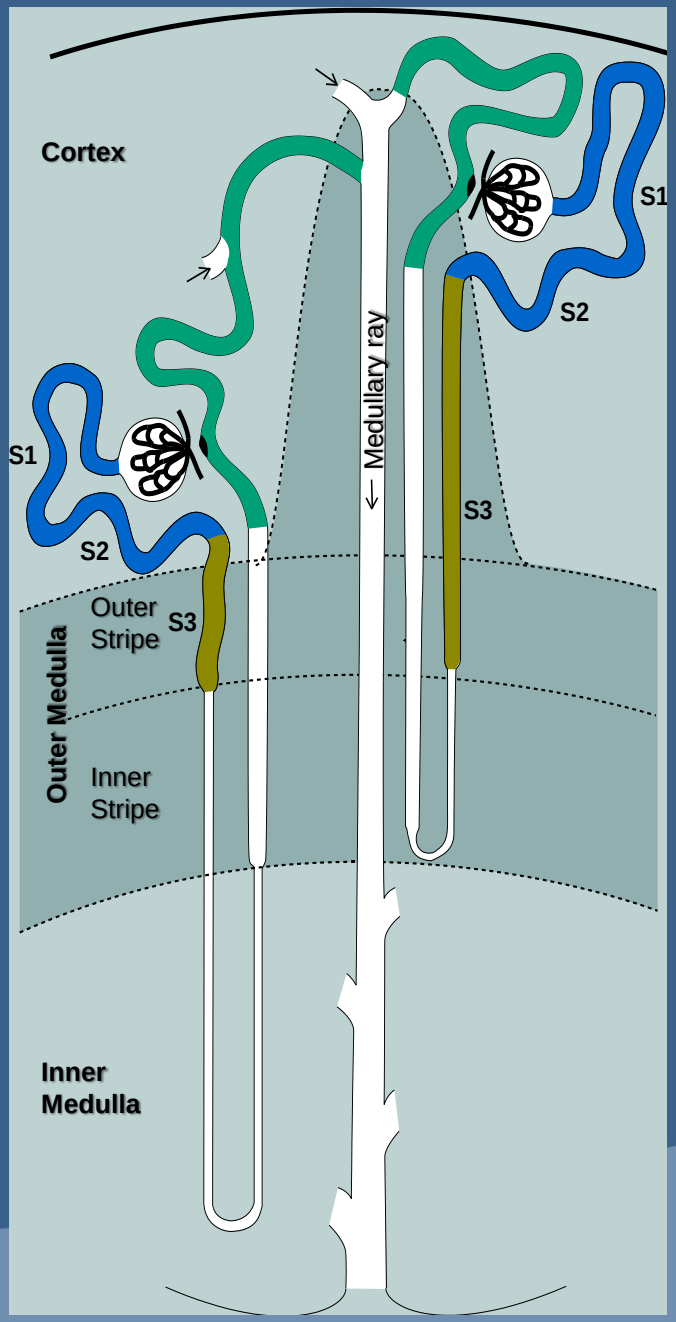
The nephron

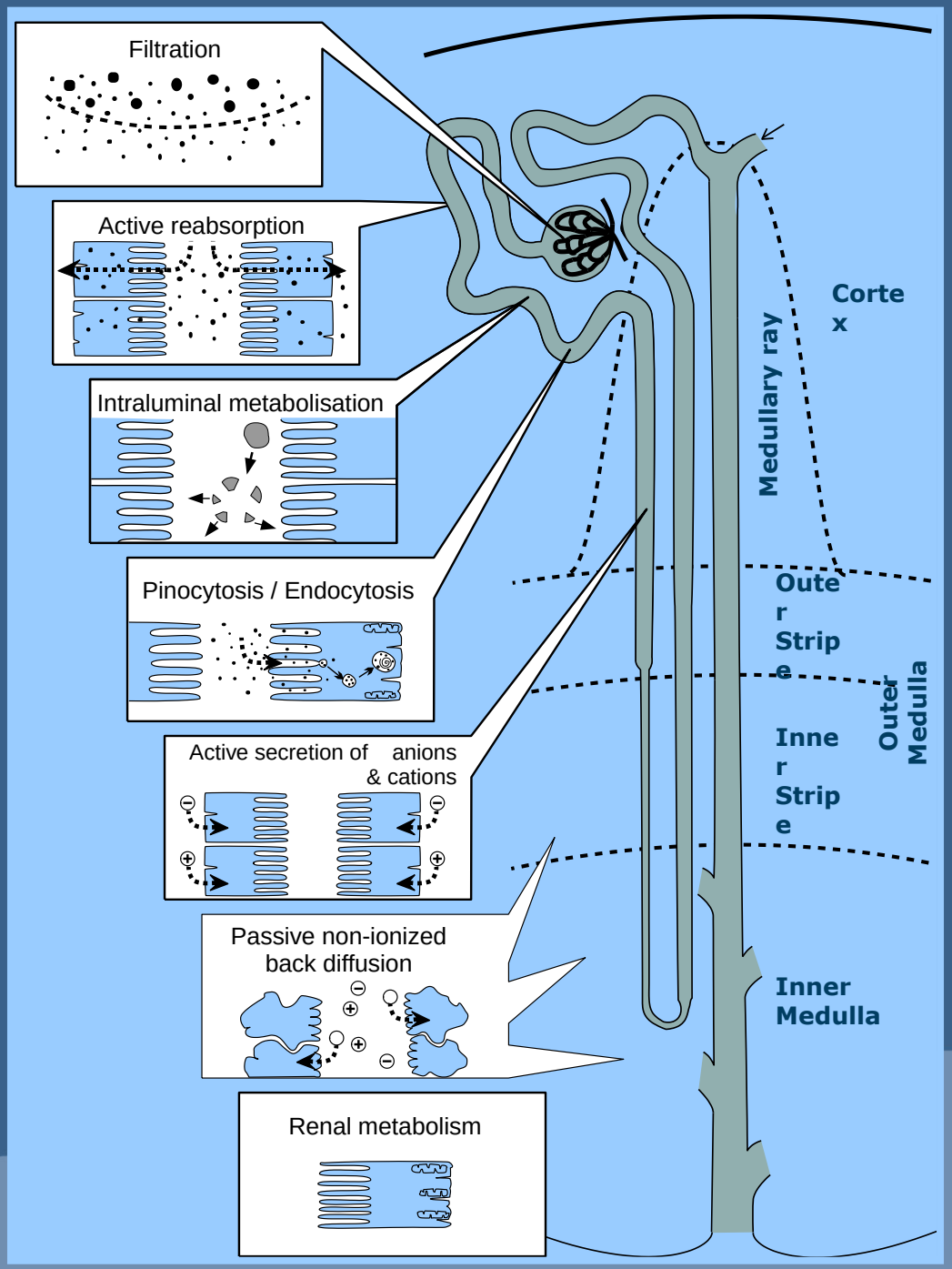




Vulnerability of the kidney

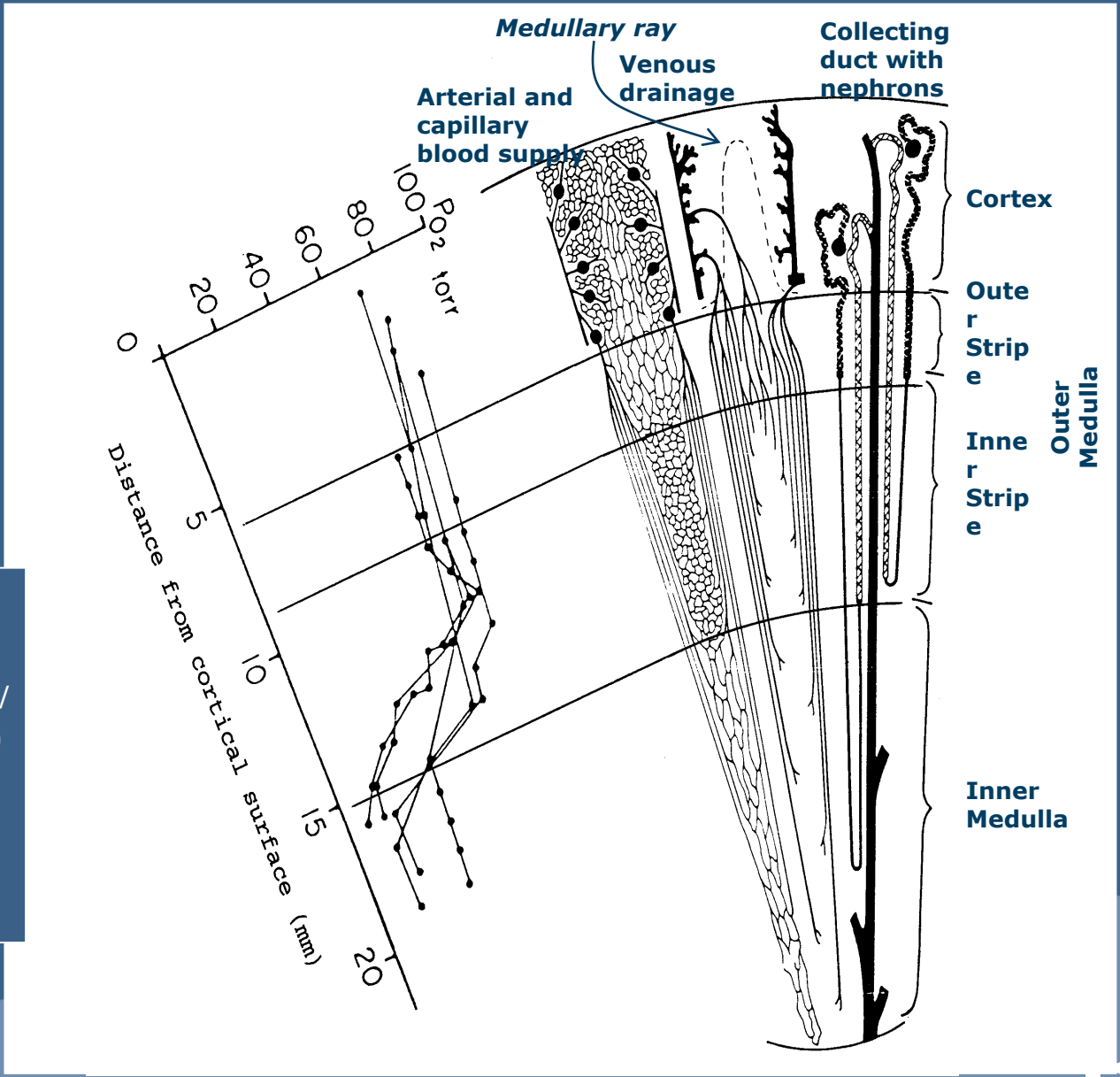
- Important blood flow (1/4 of cardiac output)
- High metabolic activity
- Largest endothelial surface by weight
- Multiple enzyme systems
- Transcellular transport
- Concentration of substances
- Protein unbinding
- High O_2 consumption/delivery ratio in outer medulla

Types of renal handling



De Broe ME & Roch-Ramel F: in 'Clinical Nephrotoxicity', Ed. De Broe, Porter, Bennett, Verpooten. Kluwer Acad. Publ., Dordrecht, 1998

Blood oxygen pressure and blood supply in the kidney



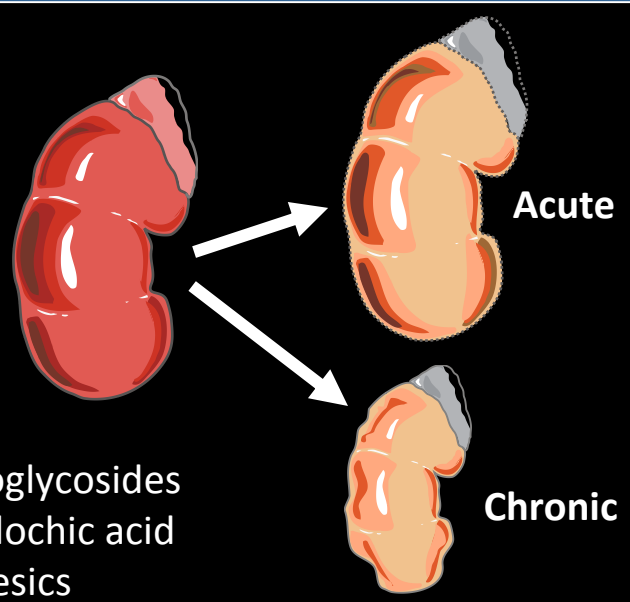
Adapted from:
Brezis M et al:
"The Kidney",
4th Ed.
(Brenner & Rector)
Saunders, 993-1061,
1991

O_2 consumption in different organs

	O_2 consumption/ O_2 delivery (%)
Kidney	08%
Renal outer medulla	79%
Brain	34%

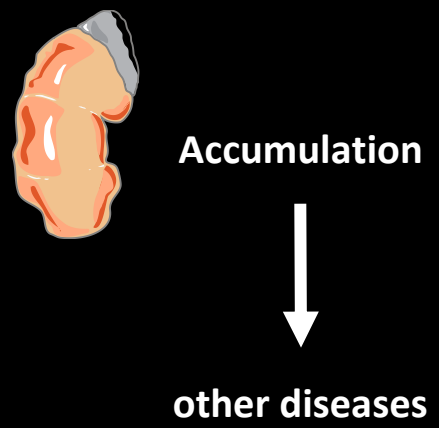
Nephrotoxicity – Phenotypes

A



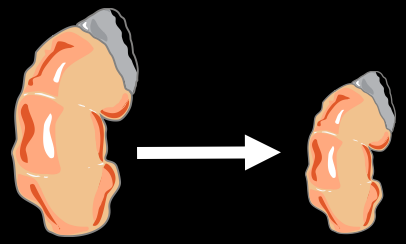
- Aminoglycosides
- Aristolochic acid
- Analgesics

B



- Aluminum
- Digoxine
- Star fruit

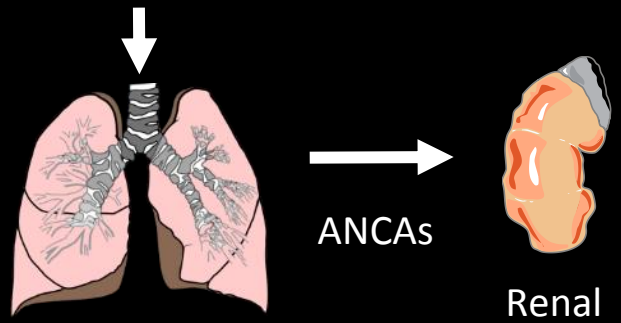
C



Accelerates progression of renal failure

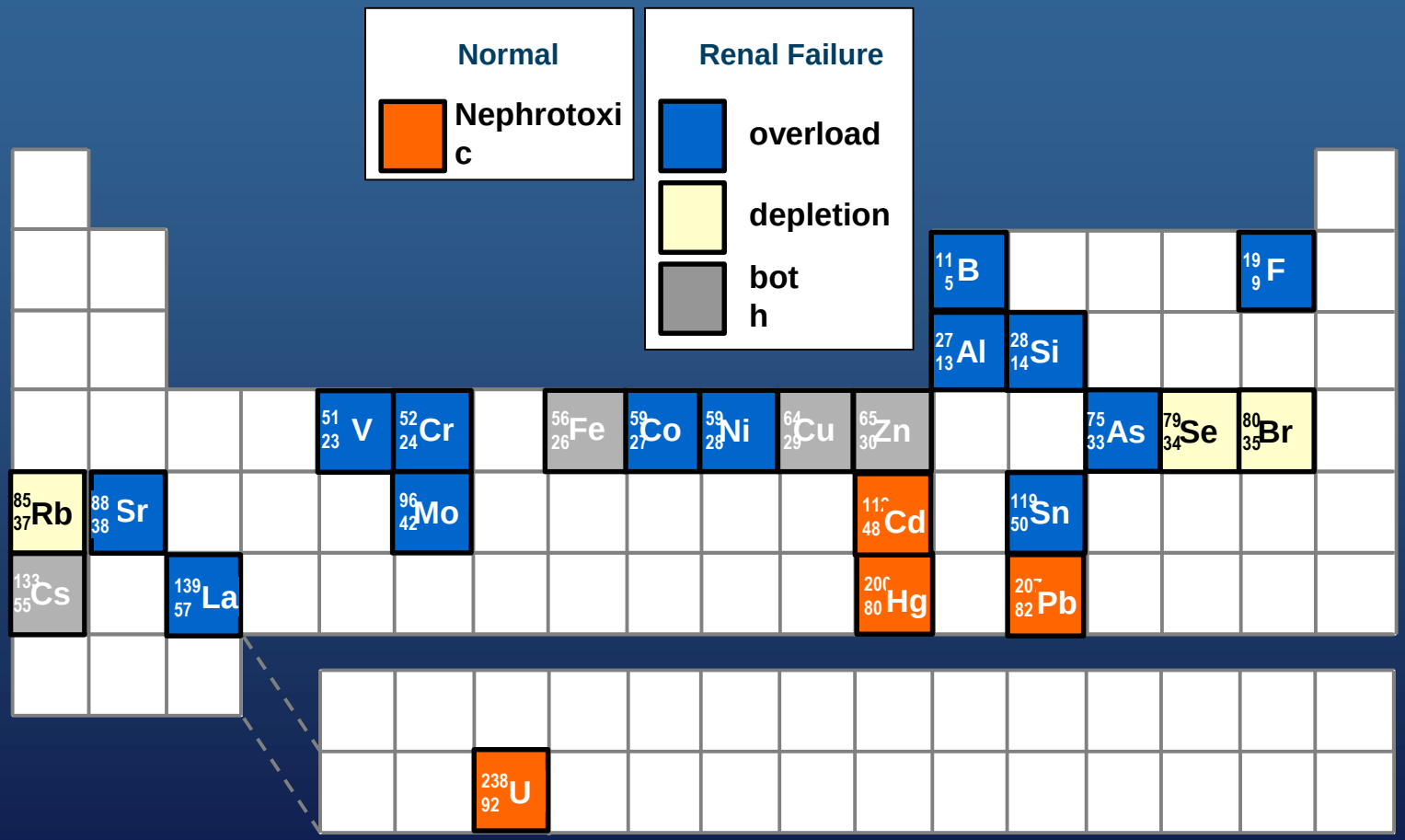
- Smoking
- Analgesics (?)
- NSAID

D



- Silica containing compounds

Mendeljev's Table and the Kidney



"It was in August 1994, I reported for duty as a consultant physician in General Hospital, Anuradhapura where I was looking after ward 7, 4A and Medical Intensive Care Unit. I was one of the two physicians attached to the hospital and the workload was enormous but, I enjoyed my day-to-day work. As I commenced my work, I found my wards were loaded with malaria, snakebites, organophosphate poisoning, yellow oleander seeds poisoning, Japanese encephalitis, typhoid and hepatitis as outstanding problems. I took special interest in managing these diseases. However, in 1995, there were sporadic cases of CKD inviting my special attention. **Then, I realized numbers of CKD were increasing by 1996. In early 1997, I carried out an audit on CKD related deaths in my male medical ward. The results astonished me as more than 100 patients have died in 1996 due to CKD in my ward alone"**.

CKD in Rajarata surfaced in early 90s: unpublished personal experience.

June 20, 2015, The Island newspaper

S.A.M. Kularatne- Professor of Medicine, University of Peradeniya, Sri Lanka

Chronic kidney disease of unknown etiology in Sri Lanka Guatemala, Nicargua ..India

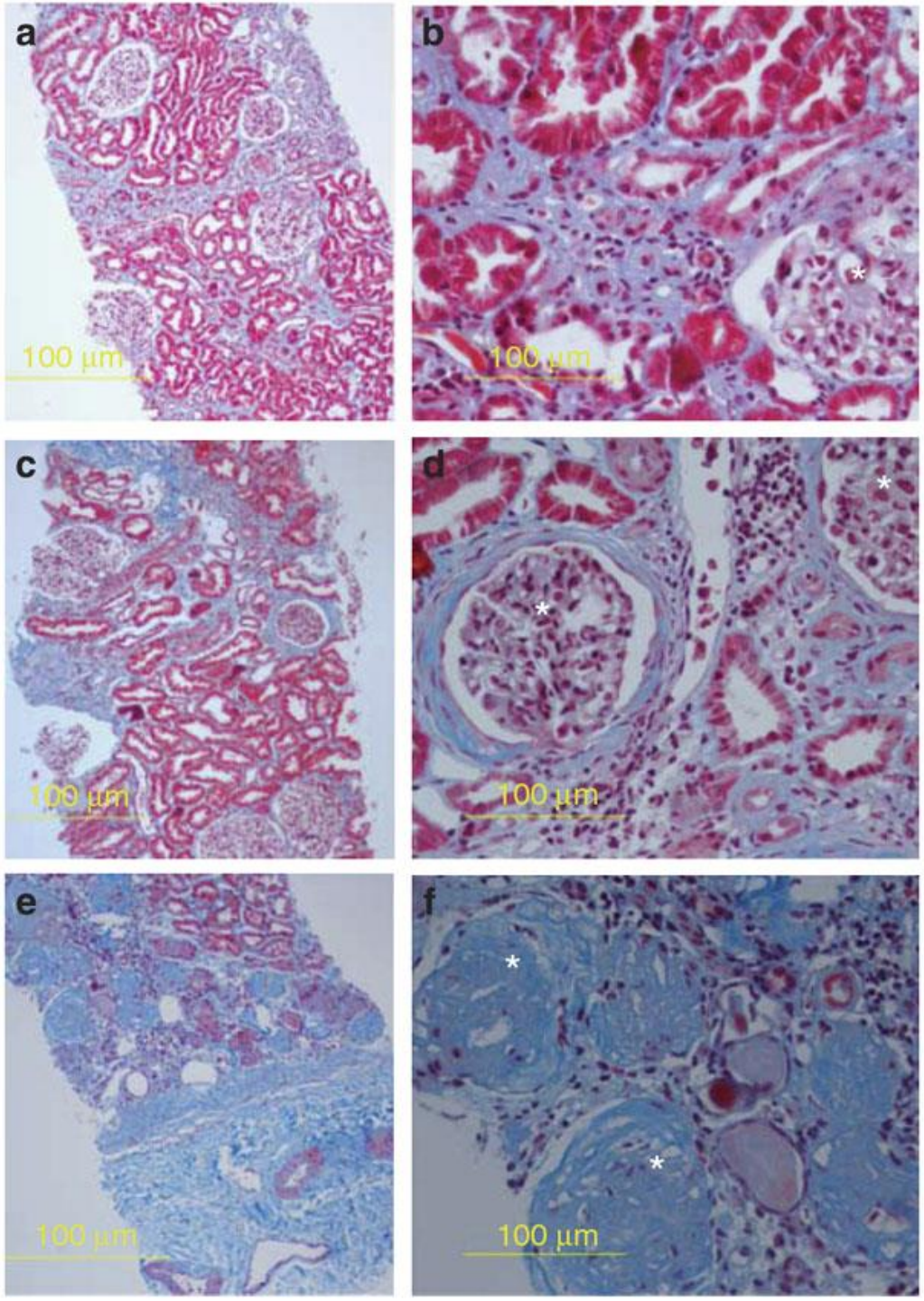
- Slow, progressive, asymptomatic development
- Age less than 20 years at start
- Men working in agriculture
- ESRF 40-60 yrs
- Anaemia
- Bilateral small kidney, loss of cortico-medullary differentiation
- Renal biopsy, tubular atrophy, fibrosis.
- Hypertension is not prevalent nor diabetes
- Ochratoxin excluded, cadmium; other environm. toxin ,pesticides ?
  - Sri Lankan traditional Ayuverdic pharmacopoeia: toxic plants ? (Crotalaria species, Cassia auriculata, Hollarrhena antidysenterica)

Chronic Interstitial Nephritis in Agricultural Communities (CINAC)

Uncertain etiologies of proteinuric-chronic kidney disease in rural Sri Lanka

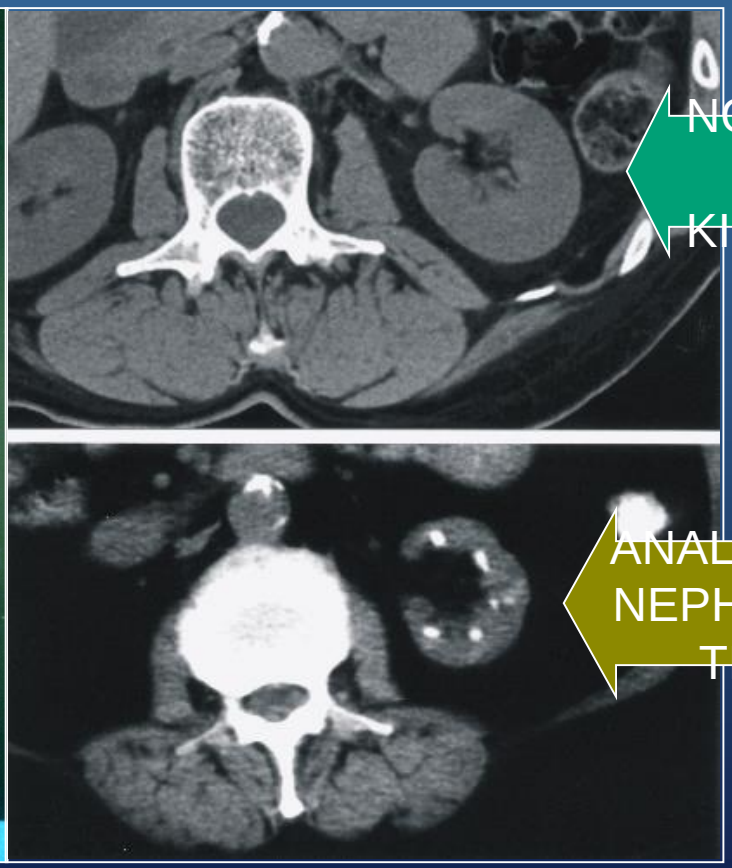
Characteristics of 264 patients

Characteristic	Medawachchiya N=130	Yatinuwara N=68	Hambantota N=66	P-value
Age (years)				
Range	20–90	20–76	20–85	
Mean ± s.d.	44.05 ± 20.39	52.06 ± 13.53	49.55 ± 14.71	0.006
Sex distribution				
Males	79 (58.5)	38 (55.9)	35 (53.0)	0.92
Occupation				
Farmer	92 (70.8)	06 (8.8)	24 (36.4)	<0.001
Direct exposure to agrochemicals				
Yes	101 (77.7)	05 (7.4)	23 (34.9)	<0.001
Tobacco-smoking males[a]	43 (56.7)	11 (28.9)	18 (51.4)	0.22
Alcohol consumption, males[a]	40 (52.6)	07 (18.4)	11 (31.4)	0.04
Prevalence of diabetes	05 (3.9)	50 (73.5)	28 (42.4)	<0.001
Prevalence of hypertension	68 (52.3)	44 (64.7)	23 (34.6)	<0.002
Prevalence of long-standing hypertension	07 (5.4)	42 (61.8)	17 (25.8)	<0.001
Prevalence of other etiologies	11 (8.5)	2 (2.9)	13 (19.7)	<0.01
Prevalence of uncertain etiology	109 (84)	2(2.9)	6 (9.1)	<0.001
Family history of CKD	14 (10.7)	08 (11.8)	03 (4.6)	0.33



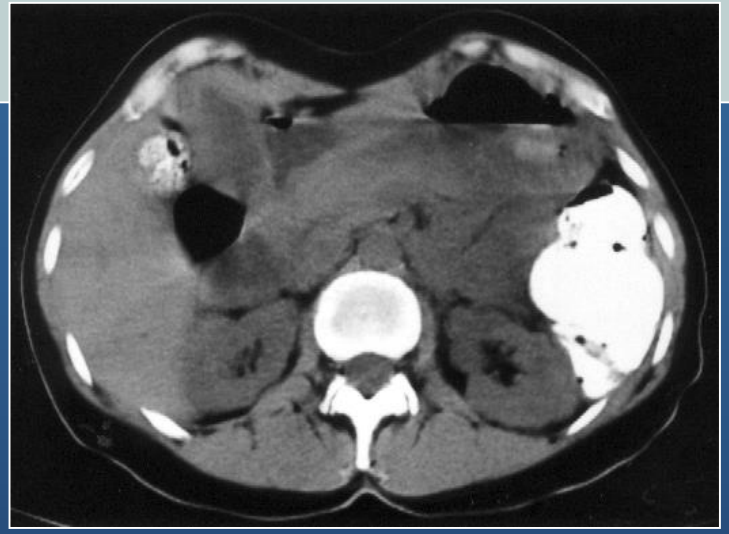
Athuraliya NT et al: Kidney Int 80: 1212-1221, 2011

Macroscopic aspect of an AN kidney (1982) and CT-scan

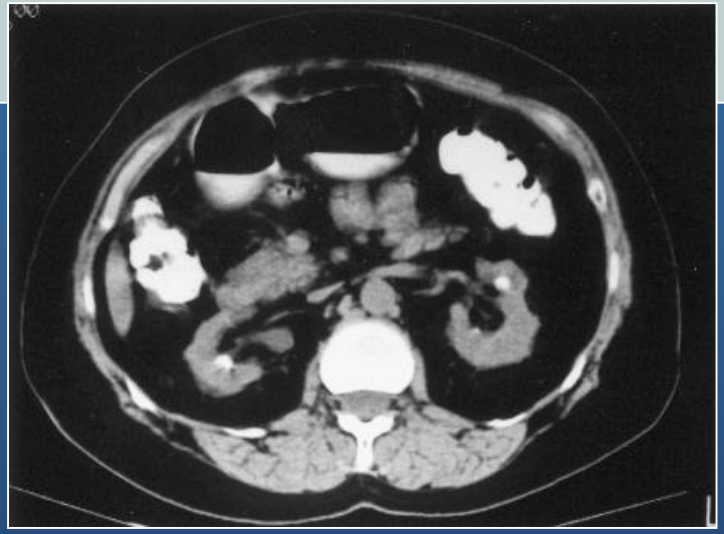


IMAGING OF CHRONIC TUBULO-INTERSTITIAL DISEASES

Chinese Herbs Nephropathy
(CrCl 46 ml/min)

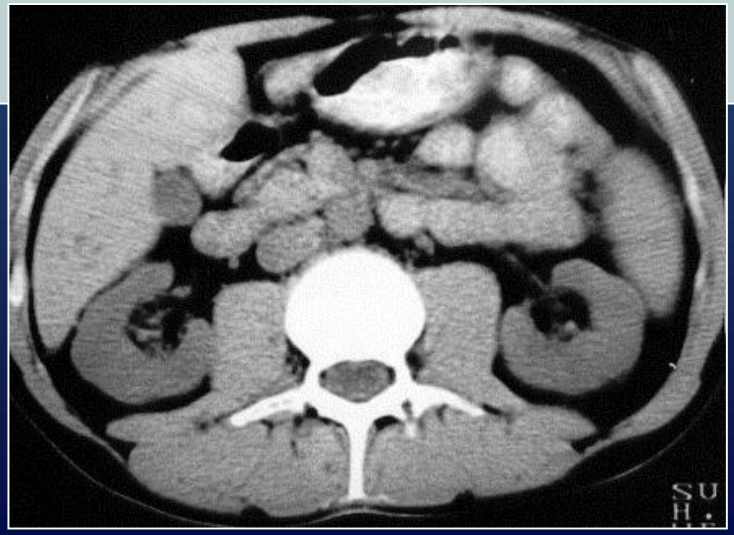


Analgesic Nephropathy
(CrCl 39 ml/min)



Balkan Endemic Nephritis
(CrCl 15 ml/min)

Chronic 5-ASA Tubulo-Int. Nephritis (CrCl 25 ml/min)

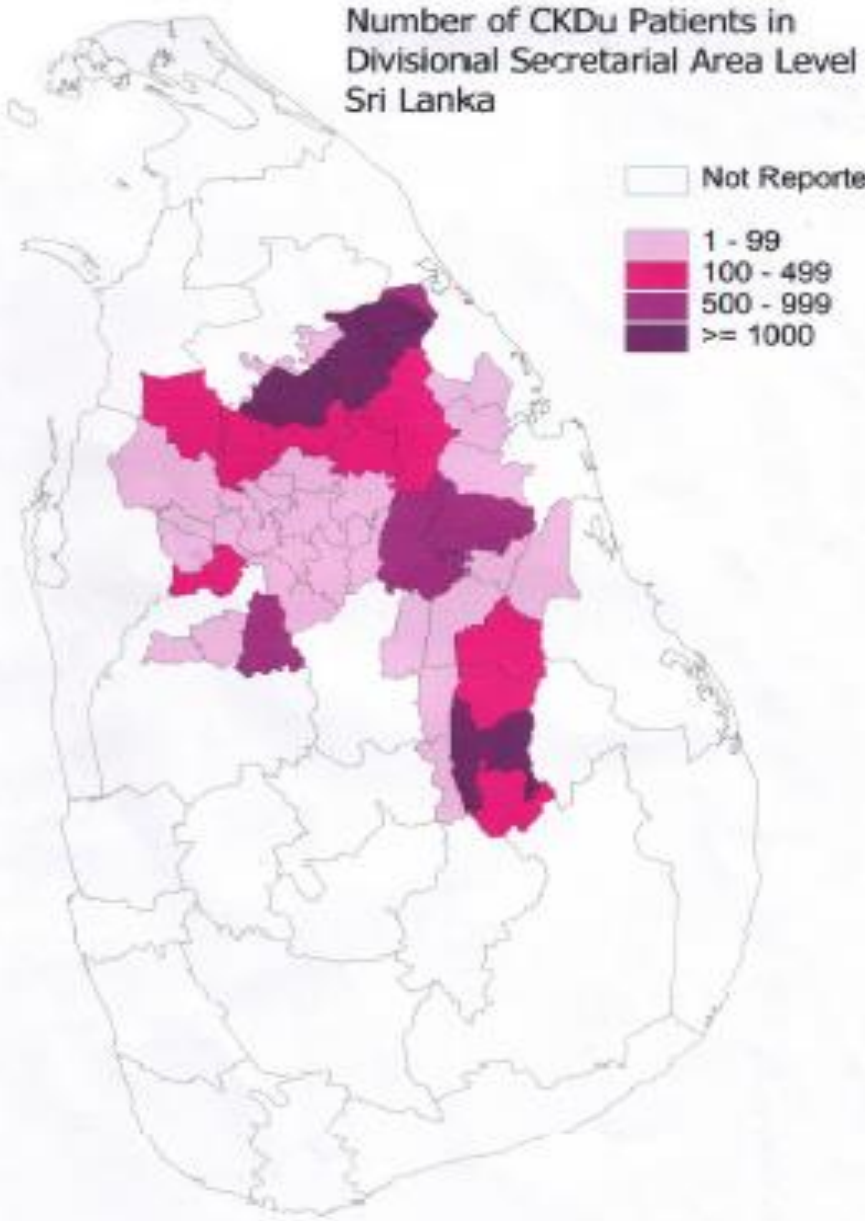


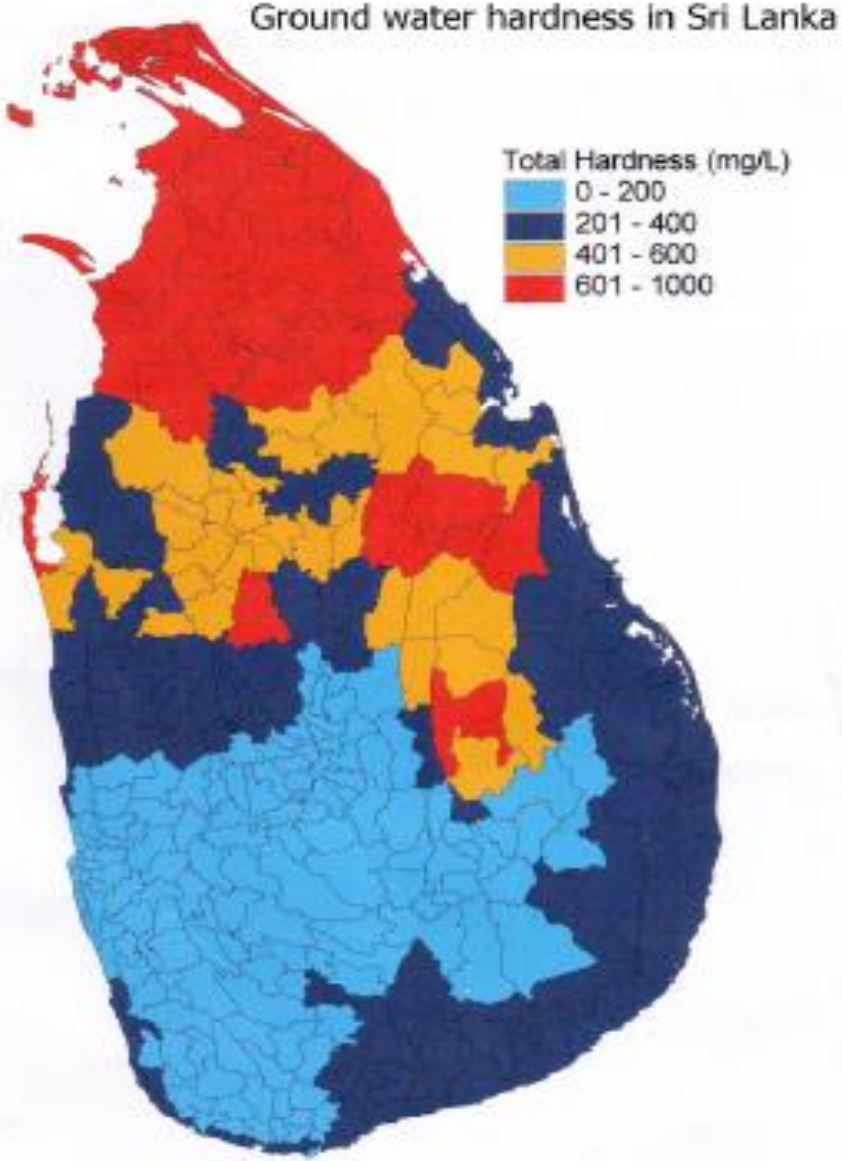
Differential diagnosis of some forms of chronic interstitial nephritis

	Chinese Herb Nephr.	Balkan Endemic Nephr.	Analgesic Nephropathy
	Aristolochic Acid Nephropathy		
Etiology	Aristolochic acid		Analgesics + addictive substances
Course towards ESRF	6 months – 2 years	> 20 years	> 10 – 15 years
Kidney imaging	Shrunken Irregular contours No calcifications	Shrunken Smooth surface No calcifications	Shrunken Irregular contours Papillary necrosis Calcifications
Histology • Cellular infiltration • Fibrosis • Atrophy	 + ++ ++	 + ++ +++	 ++ ++ ++
Capillarosclerosis	+	+	+
Apoptosis	++	++	?
Urothelial malignancies	+ upper tract	+ upper tract	+ (*)
Familial occurrence	–	+	–

(*) as long as phenacetin was part of the analgesic mixtures

Kidn . Intern . 2012





Geographical distribution of patients with CKDu and ground water hardness in Sri Lanka. Ground water hardness data- with the courtesy of Water Resources Board of Sri Lanka. Much less use of agro-chemicals in Northern pro

Major changes in life style/ agricultural pattern/ climate in dry zone Sri Lanka prior to emerging of CINAC

Life style	➢ Physical activity reduced as machines were (mainly 2 wheel mini tractor) introduced.
Agriculture	➢ **late 1960s**- Introduction of chemical fertilizers . ➢ **early 1980s**- Land expansion (365,000 ha. of land) under Mahaweli development scheme in. ➢ **earlly 1980s**- Introduction of newly developed high yield rice varieties ➢ **mid 1980s**-bigining of wide usage of herbicides – paraquat, MCPA, glyphosate
Climate	➢ No significant change

1000 BC- 1990 AD

1990 AD .....

7. Multivariable analysis found highest risk for CINAC was among participants who drank well water (OR 2.52, 95% CI 1.12-5.70) and had history of drinking water from an abandoned well (OR 5.43, 95% CI 2.88-10.26) and spray glyphosate (OR 5.12, 95% CI 2.33-11.26).

Jayasumana C, et al. Drinking well water and occupational exposure to Herbicides is associated with chronic kidney disease, in Padavi-Sripura, Sri Lanka. *Environ Health* 2015; 18; 14: 6

8. Two different studies show people in the endemic region are overexposure to multiple heavy metals and pesticide residues with compare to people in non-endemic region.

Jayasumana C et al. Simultaneous exposure to multiple heavy metals and glyphosate may contribute to Sri Lankan agricultural nephropathy. *BMC Nephrol* 2015; 16:103

Jayatilake et al. Chronic kidney disease of uncertain aetiology: prevalence and causative factors in a developing country. BMC Nephrol 2013; 14: 180

Padavi Sripura
GONAMERIYAWA
B343
B342
B60
Google

august 2016

Agriculture related toxicological origin suggests

1. Time line: number of patients is directly proportional to amount of herbicide used.
2. Known causes for CIN excluded. no sarcoidosis, no history suggestive of drug induce nephropathy (NSAIDs, 5-aminosalicilic acid, not associated with uro-epithilial carcinanoma, negative for immunofluorescence.
3. Seen among paddy farmers and their family members
4. Male predominancy
5. Not seen in the northern peninsula: although rice cultivated, from 1980 to 2009 agrochemical usage was minimum in north due to restrictions imposed by government.
6. People who drink water from spring wells in the endemic region free of disease. People who drinks water from shallow wells which are close to paddy field get disease. However these wells were used since early 1900s but CINAC reported only in 1990s.

Figure 2. Structure of glyphosate molecule and its functional groups.

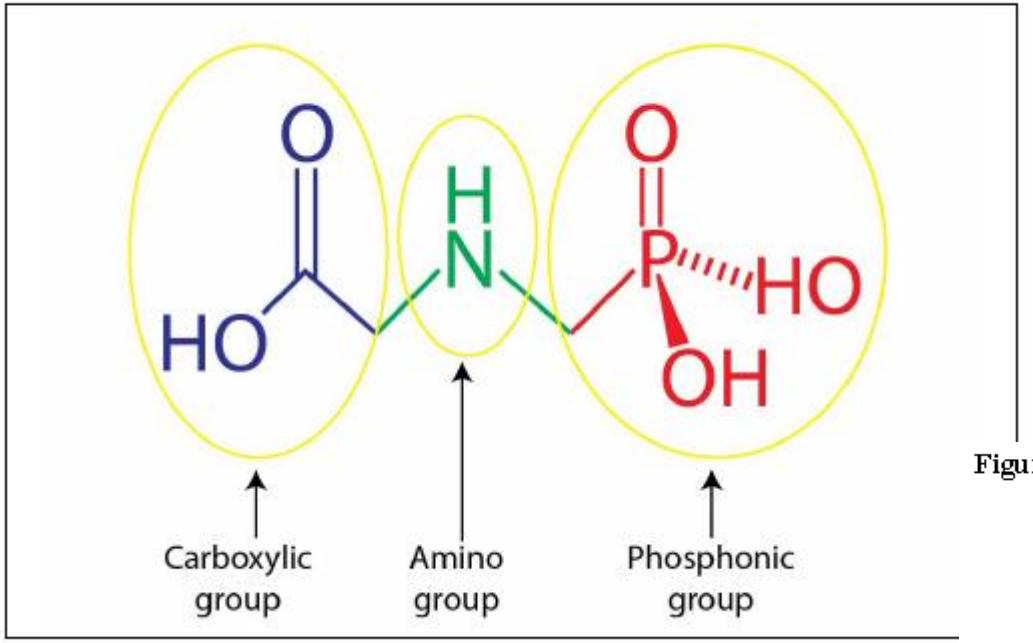
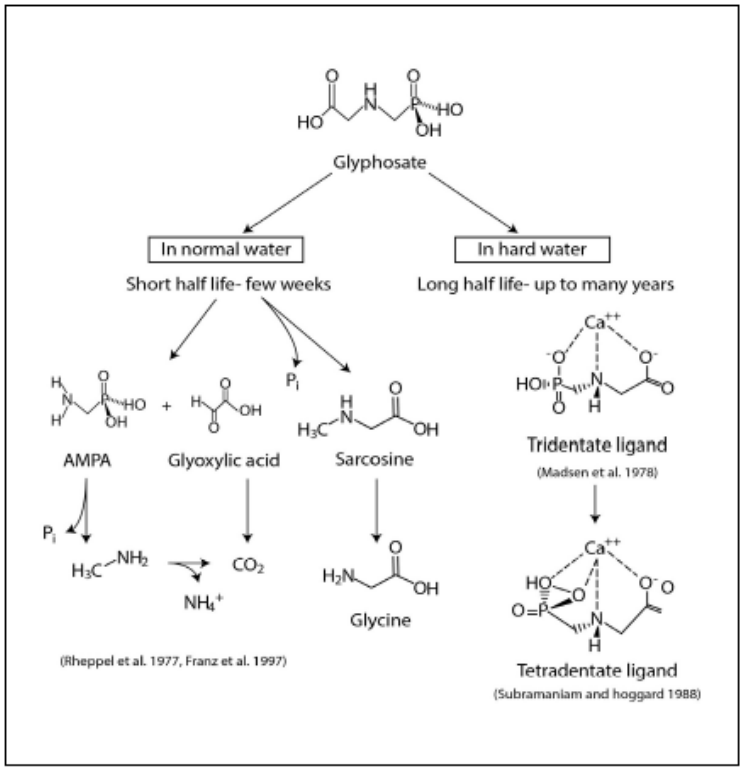


Figure 3. Degradation pathways of glyphosate in normal water and in hard water.



GLYPHOSATE,amphoteric and zwitterion structure,binding polyvalent metallic cations: Ca ,Mg, Sr, Fe, and Cd, Pb, CO ,Cr.

Glyphosate –metal complex

hosate-metal Complex (GMC)

Semi-quantitative histological analysis of renal tissue from R-treated rats

Source of renal tissue

Séralini et al. Environmental Sciences Europe 2014, 26:14)

Four groups involved in the analysis

Group 1 = control = plain water + standard diet (of the closest isogenic non-transgenic maize control)
Group 2 = dose 1 = control diet + water supplemented with 1.1 x 10-8% R (= 0.1 ppb or 50 ng/L Glyfosate)
Group 3 = dose 2 = control diet + water supplemented with 0.09% R (= 400 mg/kg Glyfosate)
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1) tubular dilatation
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3) Disrupted tubular morphology (no reliable scoring was possible)

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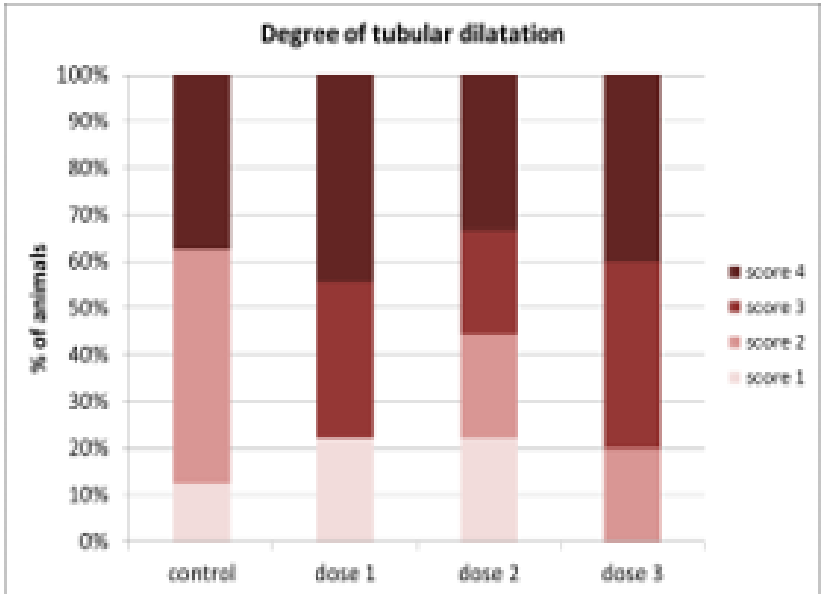
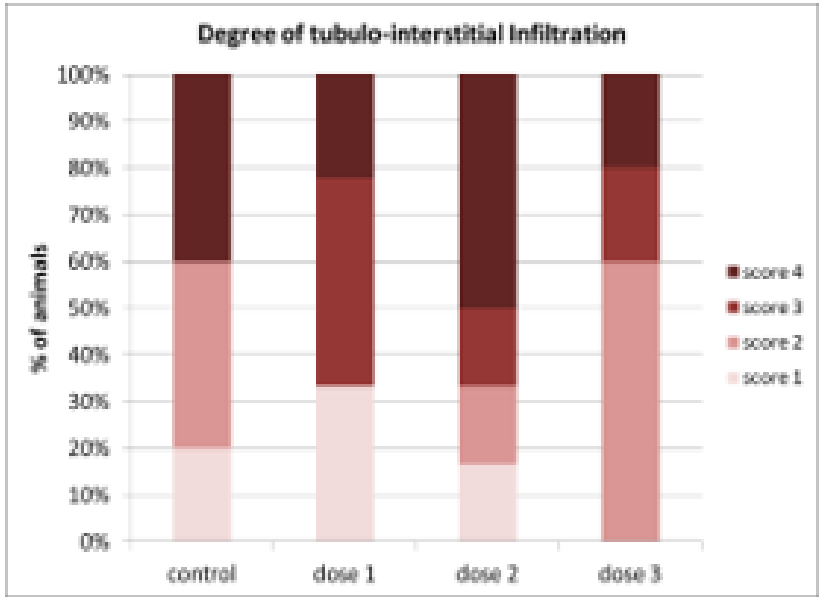
Score 1: no to mild histological feature → score 4: massive histological feature

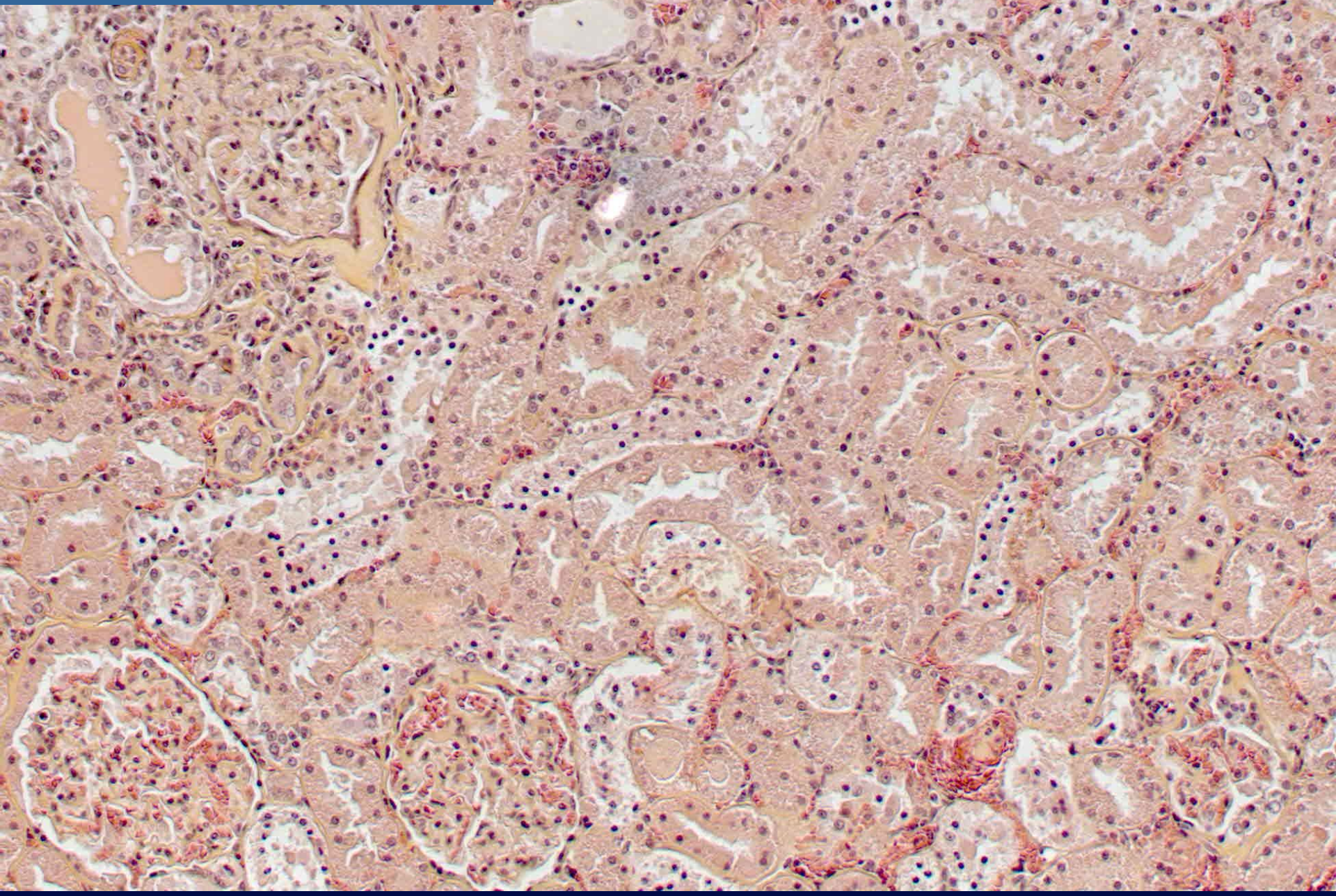
Results





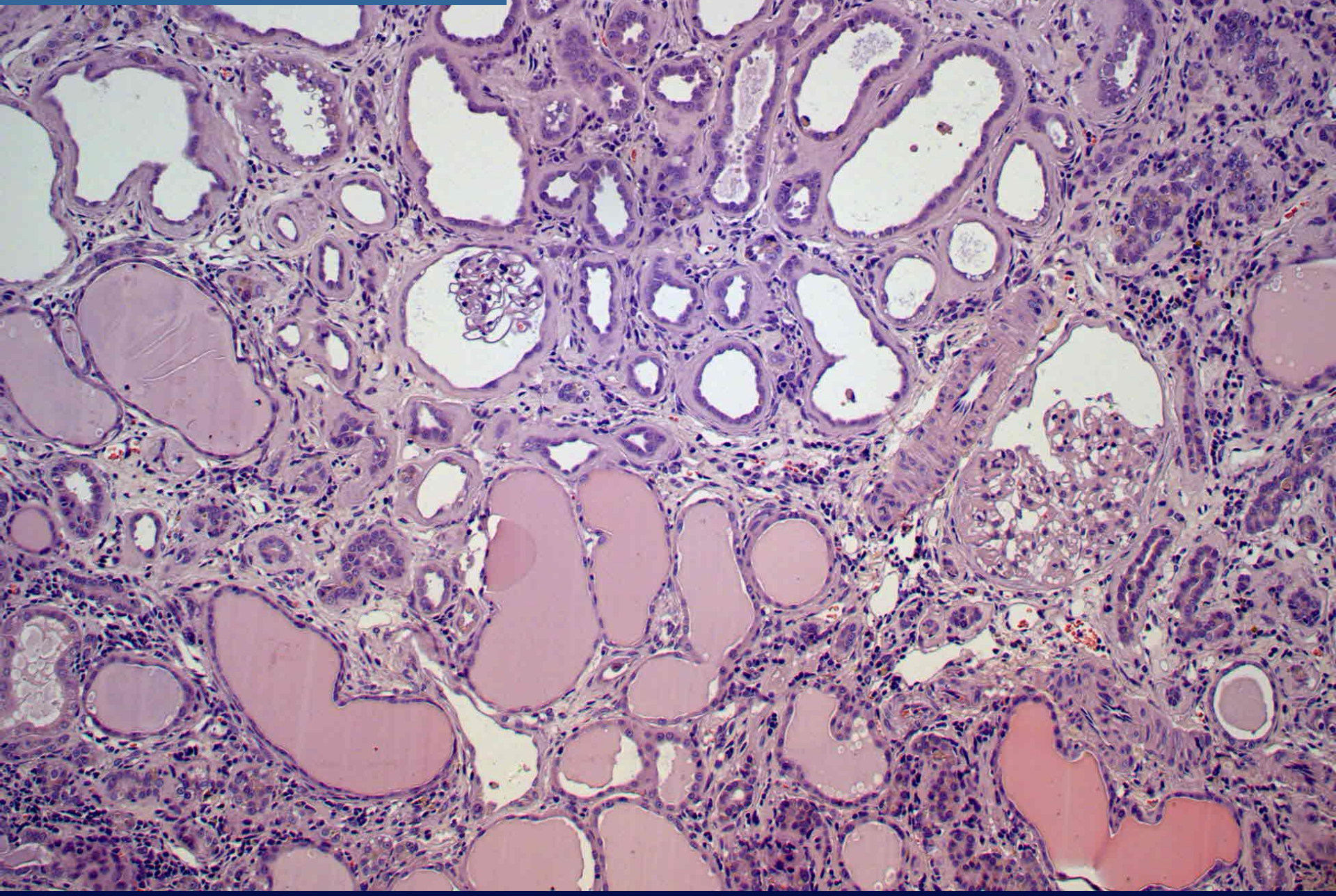
Group 1 – Control animal (8890)
-Age of death: 574d (19,1 months)
-Serum creatinine at 18 months: 7.2 mg/ml

-image 1
-magn: 16

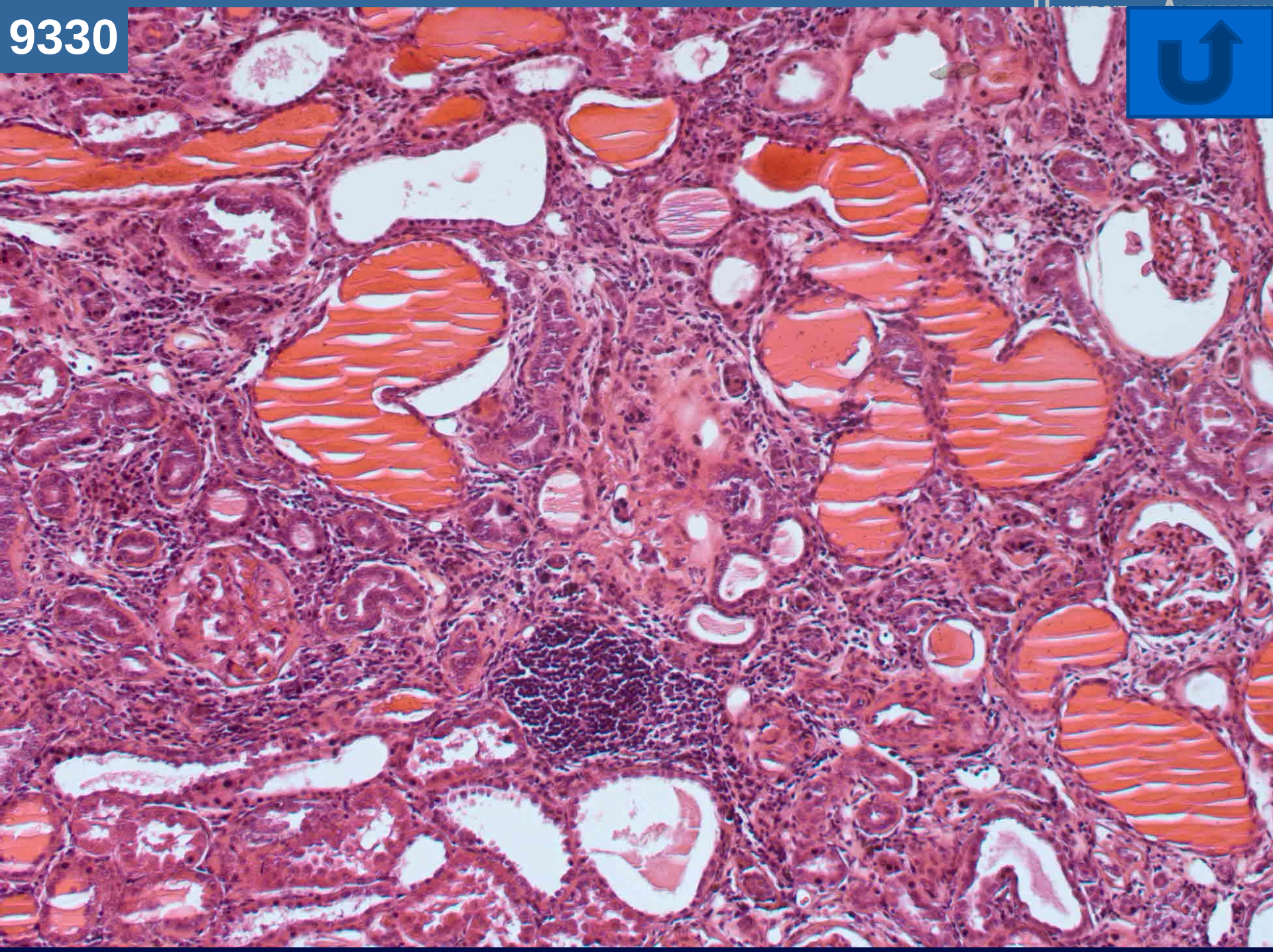


Group 1 – Control animal (9331)
-Age of death: 604d (20,1 months)
-Serum creatinine at 18 months: 10.4 mg/ml

-image 1
-magn: 10

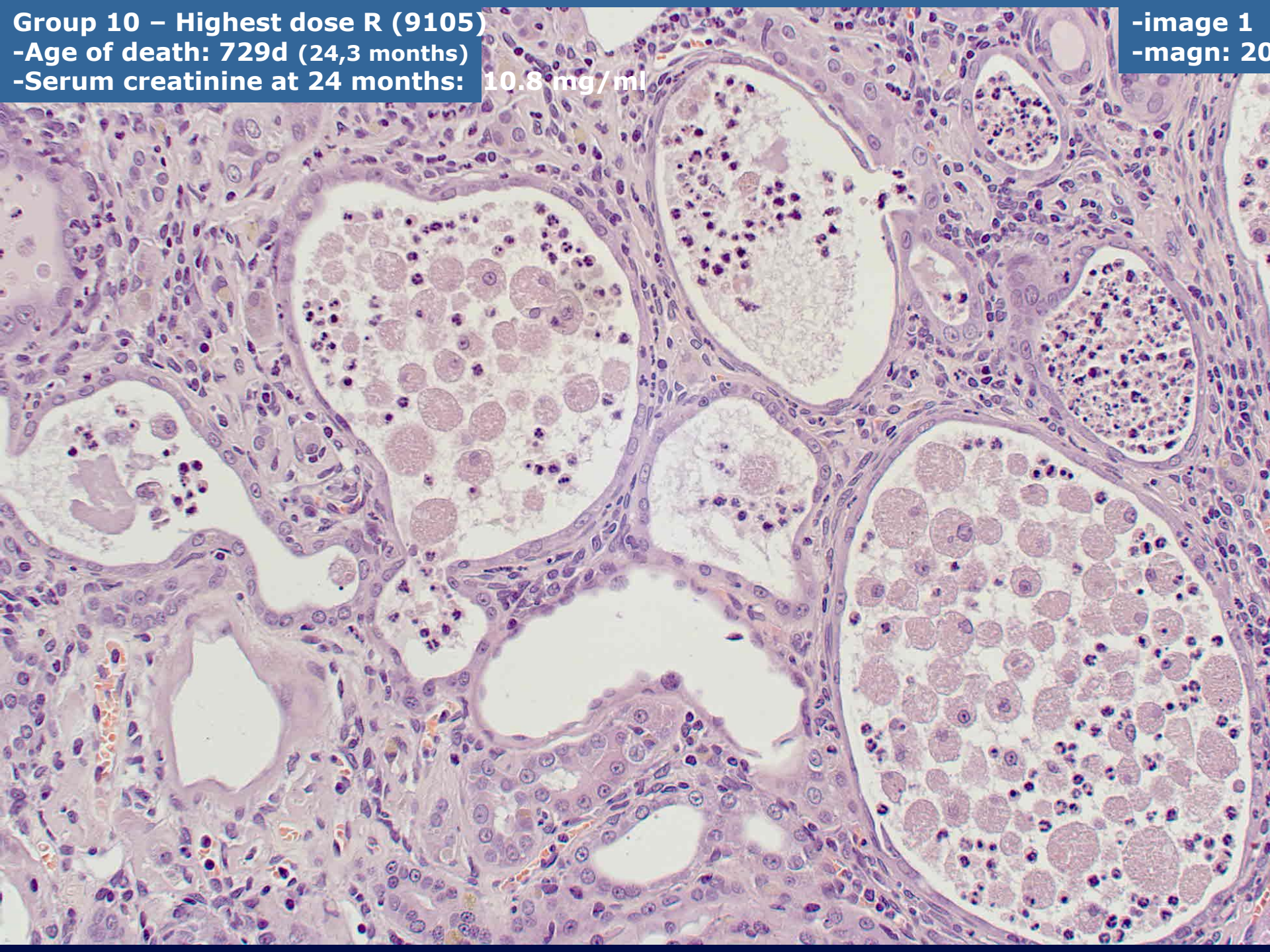


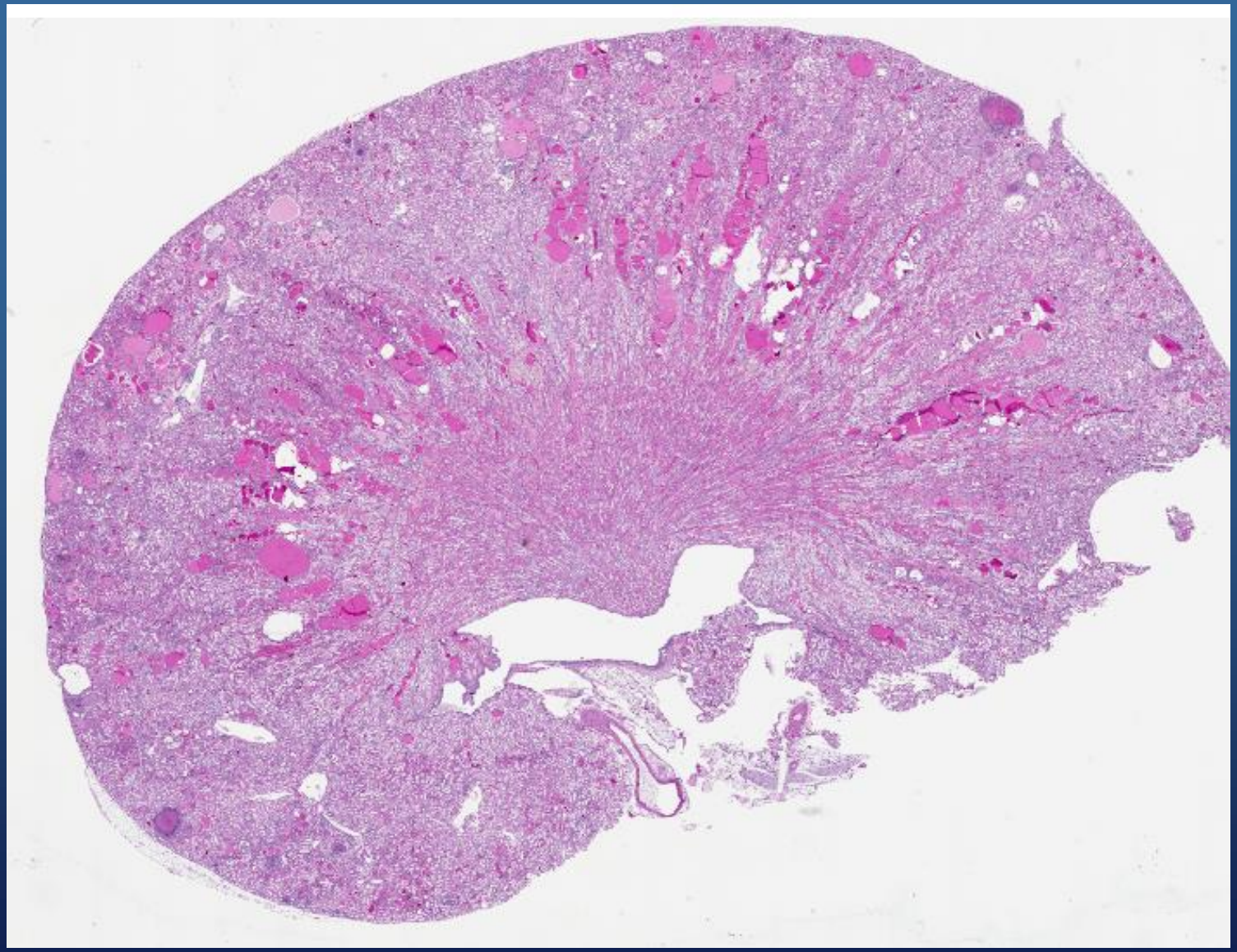
9330



Group 10 – Highest dose R (9105)
-Age of death: 729d (24,3 months)
-Serum creatinine at 24 months: 10.8 mg/ml

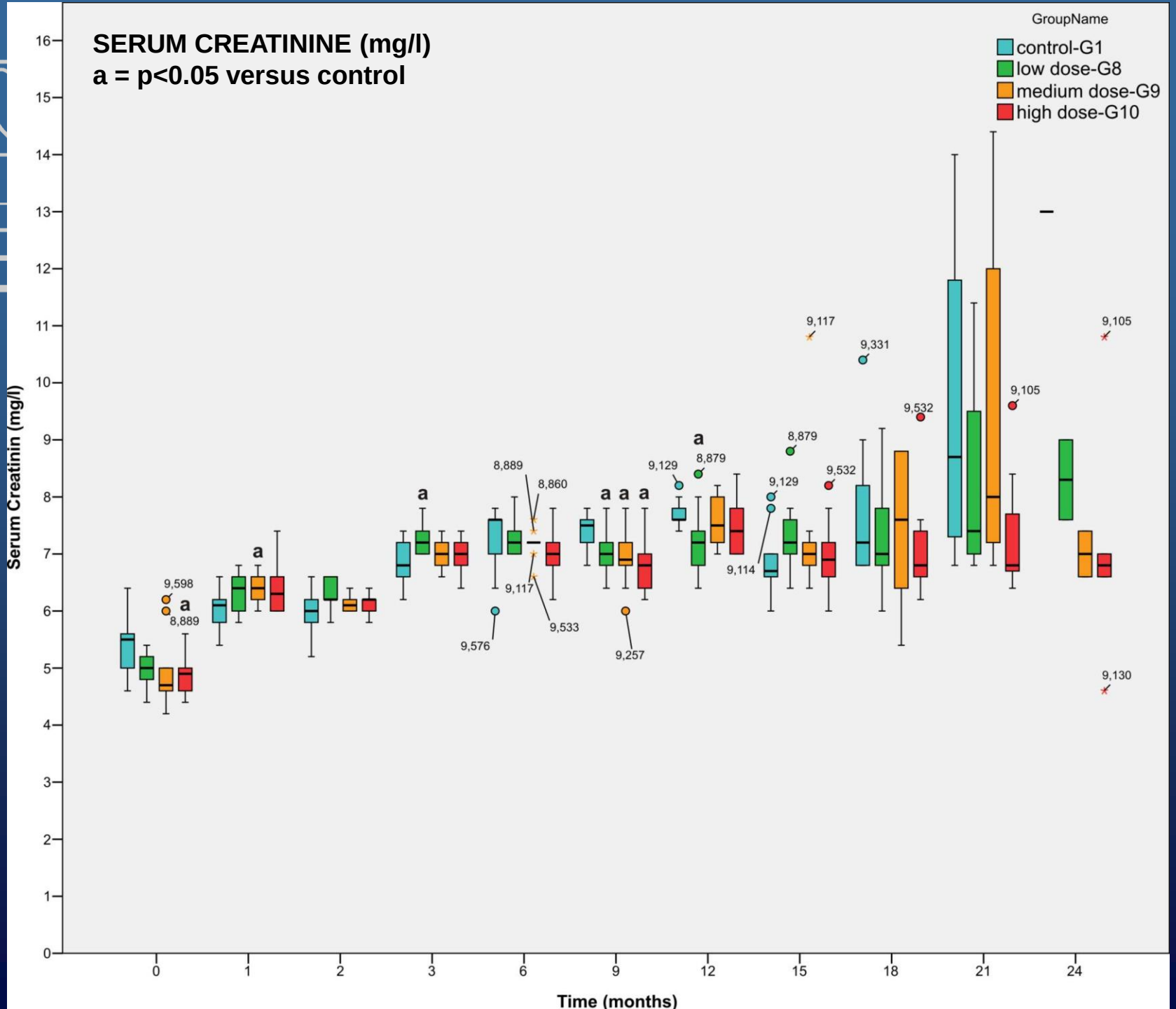
-image 1
-magn: 20

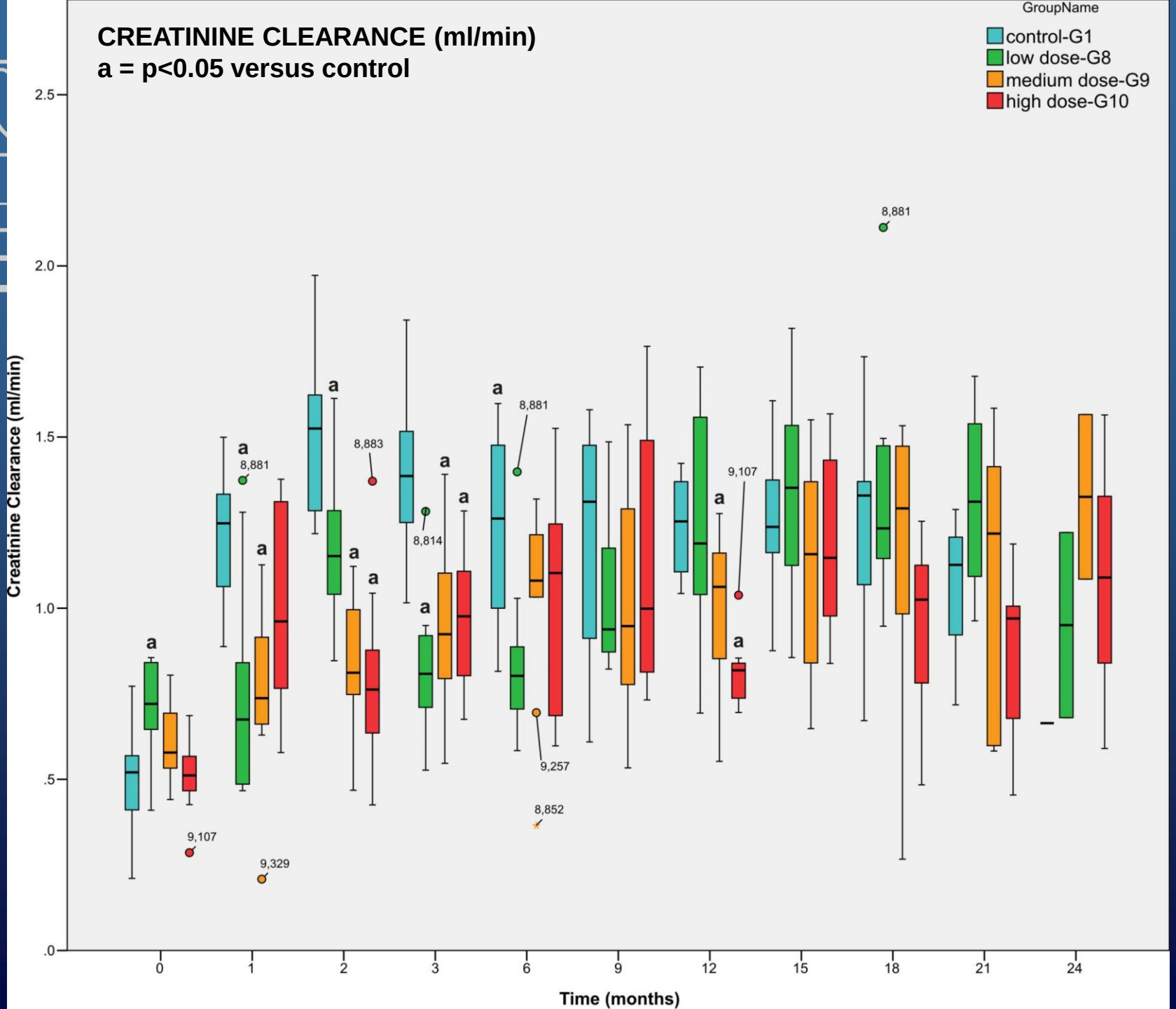


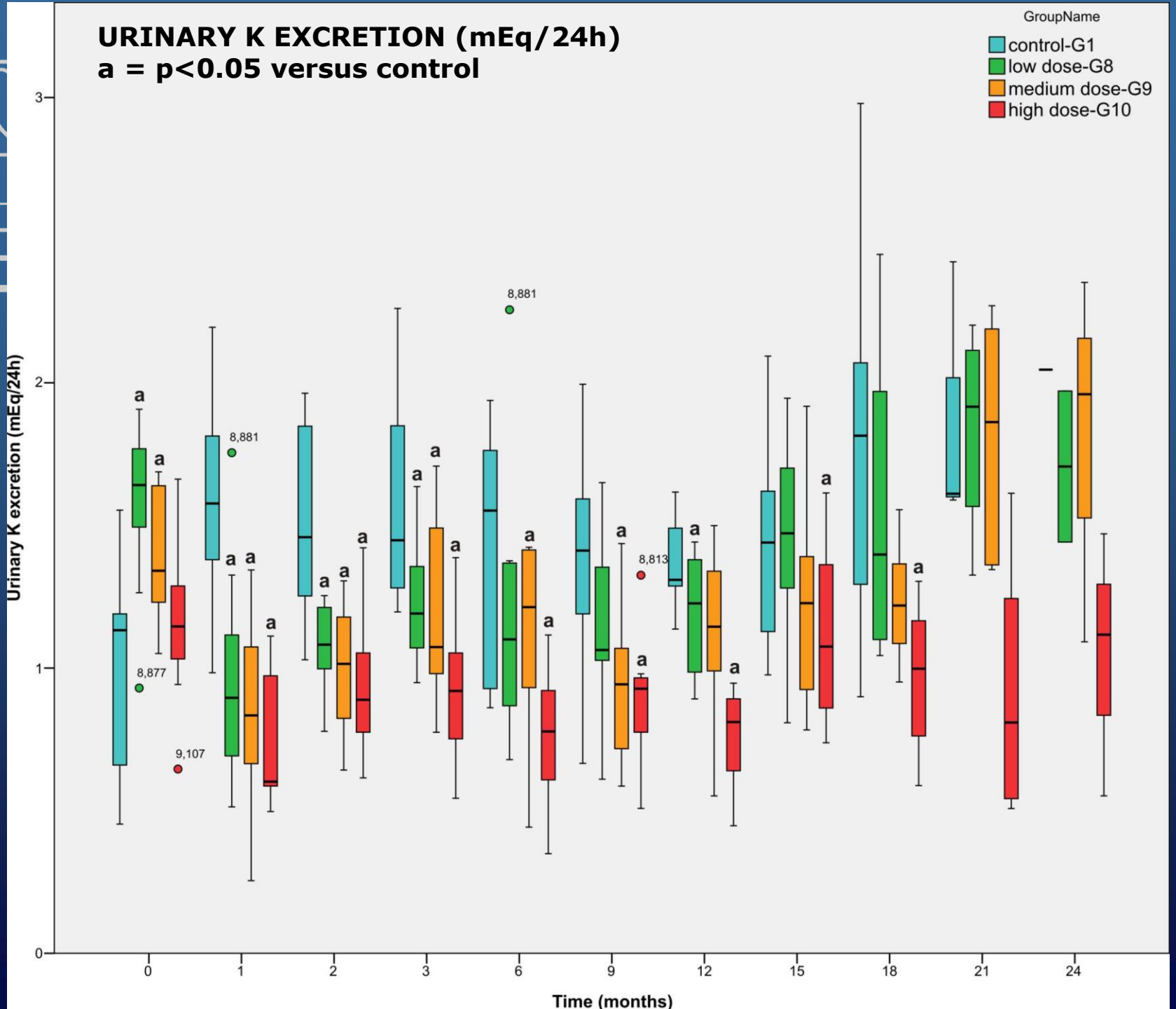


Glenda Gobe March 2016

Microcystic disease


SERUM CREATININE (mg/l)
a = p<0.05 versus control
GroupName
control-G1
low dose-G8
medium dose-G9
high dose-G10
Serum Creatinin (mg/l)
Time (months)
0
1
2
3
6
9
12
15
18
21
24
9,598
8,889
9,576
9,117
8,889
8,860
9,533
9,257
9,129
8,879
9,114
9,129
8,879
9,117
9,532
9,331
9,532
9,105
9,105
9,130


CREATININE CLEARANCE (ml/min)
a = p<0.05 versus control
GroupName
control-G1
low dose-G8
medium dose-G9
high dose-G10
Creatinine Clearance (ml/min)
Time (months)
2.5
2.0
1.5
1.0
.5
.0
0
1
2
3
6
9
12
15
18
21
24
a
8,881
8,883
8,814
9,107
9,329
9,257
8,852


URINARY K EXCRETION (mEq/24h)
a = p<0.05 versus control
GroupName
control-G1
low dose-G8
medium dose-G9
high dose-G10
Urinary K excretion (mEq/24h)
Time (months)
0
1
2
3
0
1
2
3
6
9
12
15
18
21
24
8,881
8,881
8,877
9,107
8,813
a

Semi-quantitative histological analysis of renal tissue from R-treated rats

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Séralini et al. Environmental Sciences Europe 2014, 26:14)

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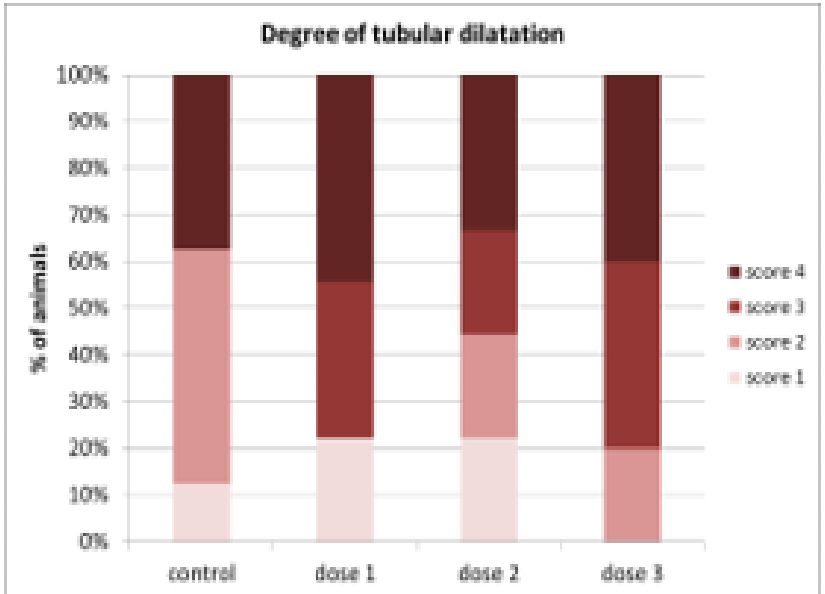
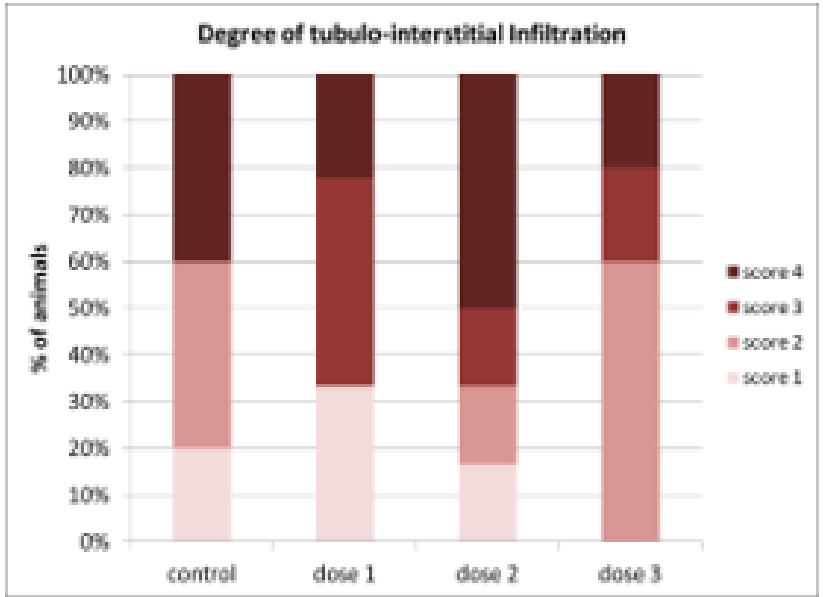




Nephrotoxic effects of Herbicides, Agrochemicals

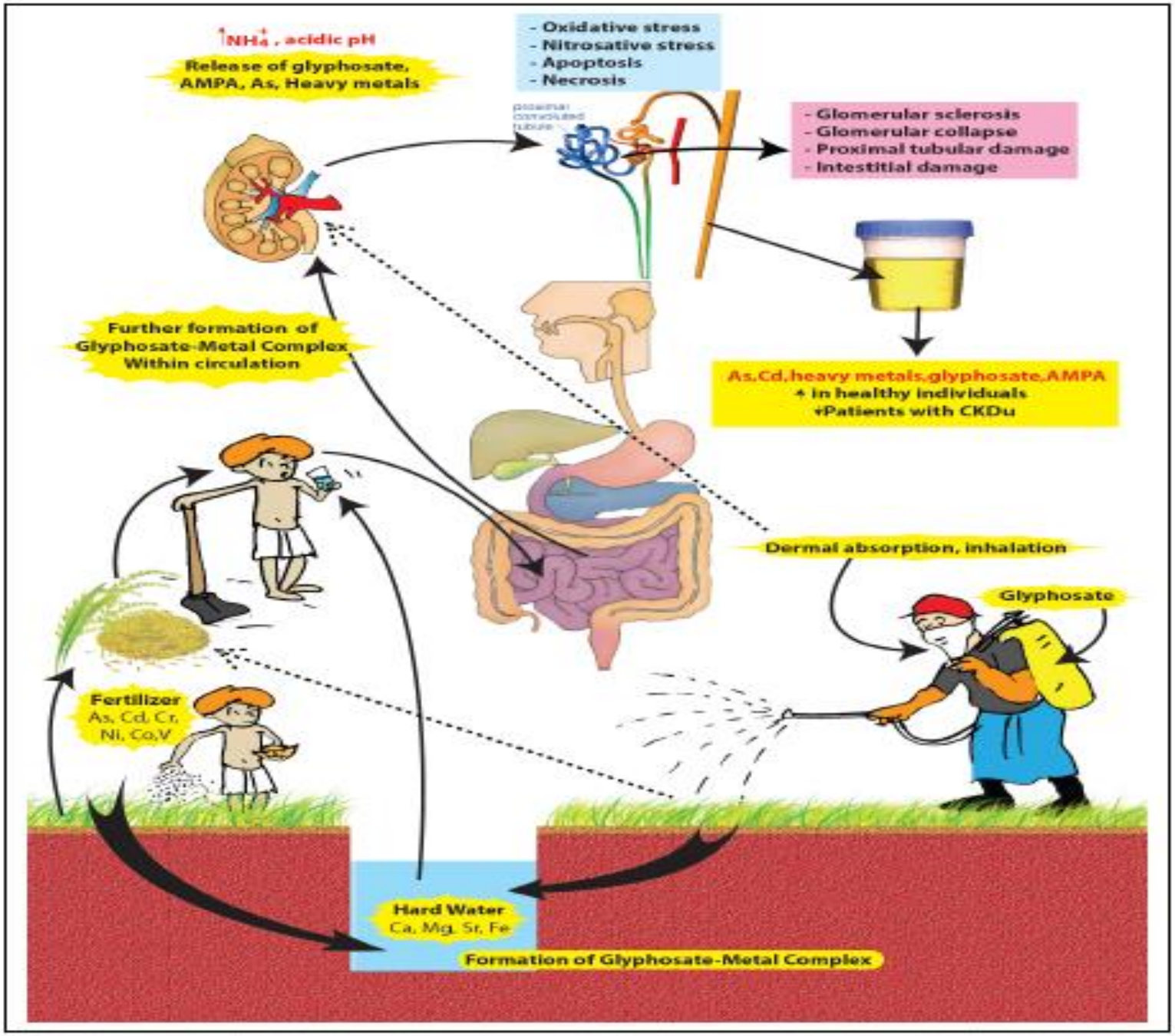
Literature is limited and not convincing

Epidemiology has some arguments

Problems multielemental, multichemical , … exposure

Water quality, chemical interactions , ways of intoxication ….

Figure 6. GMA lattice hypothesis in summary.



C Jayasumana et al Int.J. Environ. Res11: 2125-47; 2014

During the last ten years it has become evident that an epidemic of CKD affecting agricultural workers in the Mesoamerica. At an early stage the kidney disease is characterized by a lowered glomerular filtration rate (GFR) but no, or limited, albuminuria. It mainly affects men that have been working for years with hard physical work in hot climate prone to repeated episodes of dehydration and, as result of this, repeated subclinical acute kidney injuries. Cofactors for the development of the

disease, such as consumption of NSAID and large amount of fructose rich fluids, and genetic predisposition possibly exist. Severe and terminal CDK may develop. Thousands of inhabitants along the Pacific coast possibly are affected. Histopathological examination of renal biopsies shows glomerular and interstitial changes that are compatible with repeated episodes of ischemia. Reports from Sri Lanka indicate that agricultural workers in certain areas of the island may develop CKD of a similar type.

C G Elinder SCIforshen 2015

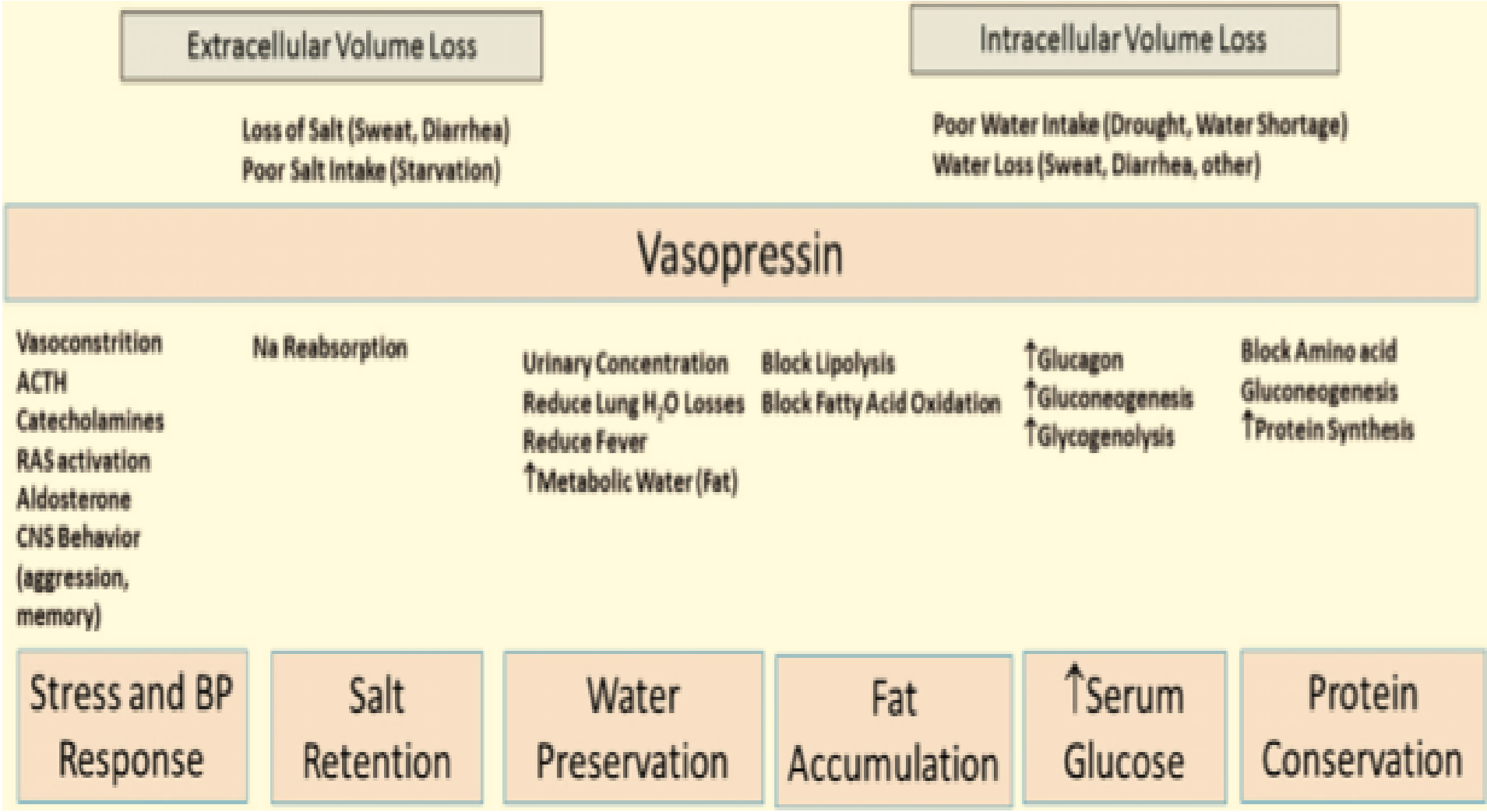
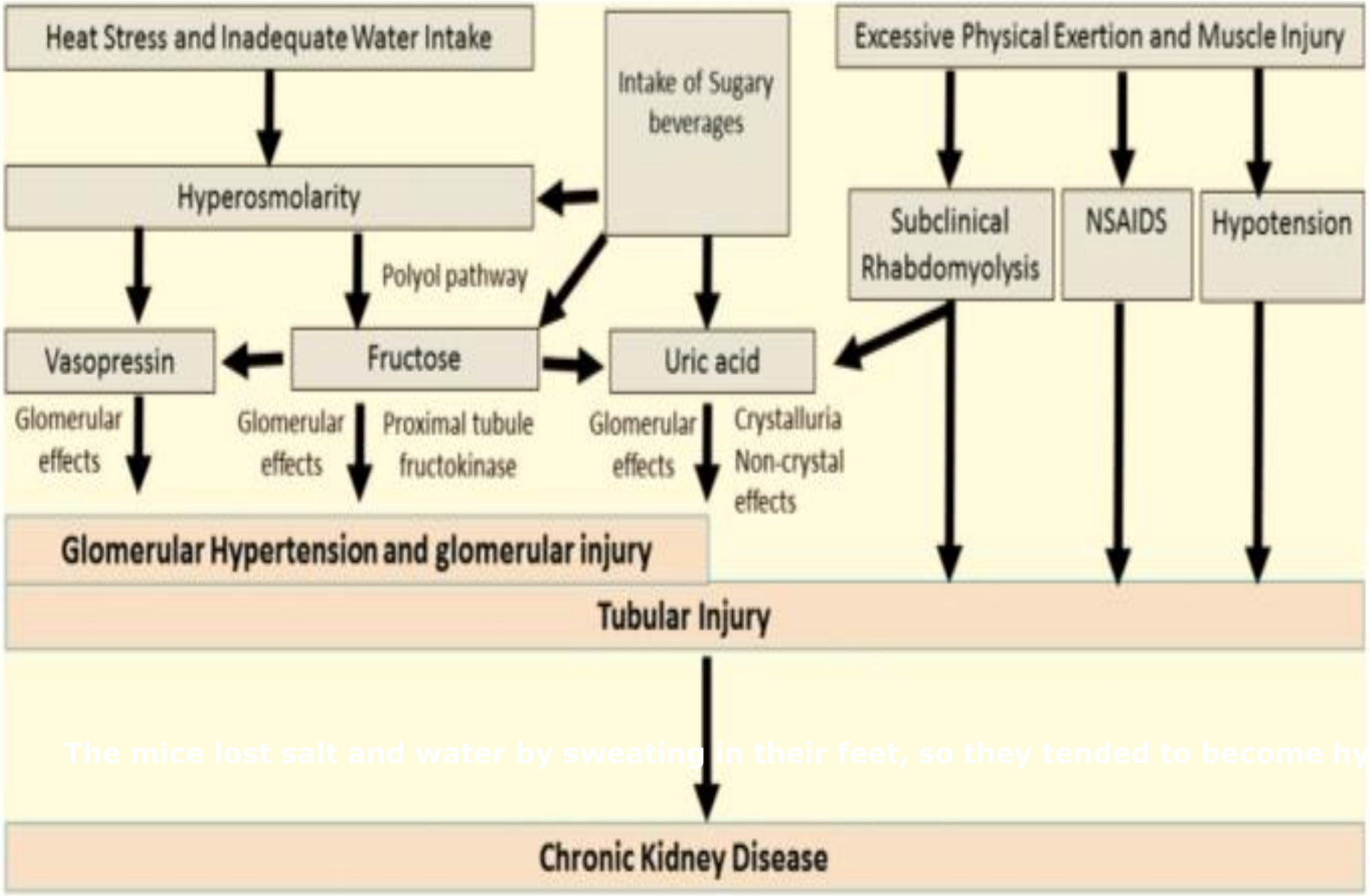


Figure 1. Vasopressin, the ultimate survival hormone. Vasopressin may have originated as a survival hormone for situations where the organism suffered from either extracellular volume or intracellular volume loss. The effects of vasopressin include actions much greater than simply preventing the loss of water but also, include generating a stress response, increasing BP, stimulating protein synthesis, stimulating fat accumulation, and maintaining elevated serum glucose (insulin resistance) to provide energy to the brain. ACTH, adrenocorticotrophic hormone; CNS, central nervous system; RAS, renin angiotensin system.



The mice lost salt and water by sweating in their feet, so they tended to become hy

Mechanisms in Heat Stress JASN Aug 2016 R Johnson et al

Is heat stress - dehydration the main trigger of CINAC?

- CINAC 1990's – herbicides – agrochemicals
- Mosaic pattern of CINAC in Sri Lanka : N NC provinces
- CUBA registry Myanmar (?)
- Woman children CINAC
- Worldwide heat stress working conditions
- Extrarenal manifestations

Contributing factor - not sufficient to cause CINAC

Nephrol. Dial. Transp. 2016 in press

Hot Jobs

- Indoor- Iron and steel foundries, brick-firing and ceramic plants, glass products facilities, rubber products factories, electrical utilities (particularly boiler rooms), bakeries, confectioneries, commercial kitchens, laundries, food canneries, chemical plants, mining sites, smelters, and steam tunnels.
- Outdoor- farm work, construction, oil and gas well operations
- Sugarcane cutters- Brazil, India, Thailand, Pakistan, Mexico, Colombia, Cuba, Indonesia, Philippines.

Chronic Interstitial Nephritis in Agricultural Communities (CINAC)

Minimal diagnostic criteria for CINAC

eGFR	<60 ml/min/1.73m^2
Proteinuria of tubular type*	Present
24 h protein excretion	< 30 mg / 24h
Living and working place	Agricultural community in CINAC endemic area
No other known causes for CKD	Diabetes, hypertension, glomerulopathies

Nephrol Dial Transplant Review Channa Jayasumana et al 2016 (in press)

Comparison of CINAC in Sri Lanka and Central America (1)

	Sri Lanka	Central America
Primarily among	Male paddy farmers, hot climate	Male sugar cane farmers, hot climate
Diabetes	No	No
Hypertension	No	No
Glomerulonephritis	No	No
Risk factors	Farmer, male sex, agrochemical exposure, drinking well water, from wells, a family history, history of snake bite, spraying glyphosate based herbicides	Agricultural worker, male sex, agrochemical exposure, heat stress, dehydration, agricultural work, profuse sweating during work, malaria, NSAID use
Clinical features		
Asymptomatic (early stages)	+	+
Loss of appetite	+	+
Lethargy	+	+
Backache	+	+
Insomnia	+	+
Arthralgia	+	+
Muscle ache	+	+
Cramps	+	+
Dysuria	+	+
Foamy urine	+	+
Neurological abnormalities	+	+
Senso neural hearing loss	?	+
Tibial artery abnormalities	?	+
Liver enzyme level	Normal	Normal

Nephrol Dial Transplant Review Channa Jayasumana et al 2016 (in press)

Comparison of CINAC in Sri Lanka and Central America (2)

	Sri Lanka	Central America
Urinary findings		
Hypernatriuria	+	+
Hypermagnesuria	+	+
Hyperphosphaturia	+	+
Hypercalciuria	+	+
Proteinuria	neg/trace, no active sediment	neg/trace, no active sediment
Beta2 microglobulin, NAG, NGAL	+	+
Blood		
Hyponatremia	+	+
Hypokalemia	+	+
Imaging	Bilateral small echogenic kidneys, decreased cortico-medullary ratio, irregular margins.	Bilateral small echogenic, kidneys decreased cortico-medullary ratio, irregular margins.
Histopathology		
Tubulo-intestitial nephritis	+	+
Interstitial fibrosis	+	+
Tubular atrophy	+	+
Interstitial mononuclear cell infiltration	+	+
Glomerular collapse	+	+
Fibrous intimal thickening and arteriolar hyalinosis	+	+
Immunofluorescence tests	Negative	Negative

Nephrol Dial Transplant Review Channa Jayasumana et al 2016 (in press)

Comparison of agricultural practices and environmental factors in Sri Lanka and Central America (1)

Feature	Sri Lanka	Central America (El Salvador)
Crop	Rice main season (maha) Brown millet, cowpea, maize, groundnut, sesame, vegetable in other season (yala)	Sugar cane, maize Beans, sorghum Fruits and vegetables
Land owns to	Farmers	90,000 blocks of land divided: Cooperatives, land owners
Number of days work in the field per annum	30-40 days mainly males	6 months winter cereals 6 months summer sugar cane
Average working hours per year	150-200 h	7 hours per day 200 days- 1400 h
Contribution by females	Minimal work in the field, they help during planting or seeding and during harvesting (2-3 days)	15% of the women are involved in agriculture themselves
Average temperature 8 am	27° C	27° C (coast)
Average temperature 12 pm	31° C	33° C
Average temperature 6 pm	27° C	28° C
Relative humidity	min 60%- July max 80%- December	min March: 45% max September: 74%
Annual rain fall in endemic region	1250-2000 mm	1900-2000 mm

Nephrol Dial Transplant Review Channa Jayasumana et al 2016 (in press)

Comparison of agricultural practices and environmental factors in Sri Lanka and Central America (2)

Feature	Sri Lanka	Central America (El Salvador)
CINAC first noticed in	1994	1999
Main fertilizers used in the region	Urea, potassium chloride, triple super phosphate	Ammonia sulfate and sugar cane formulae, triple super phosphate, urea
Main herbicide used in the region	Paraquat (1980s), glyphosate (1990s and after)	Paraquat, 2 4 D, glyphosate, triazines
Main insecticide used in the region	Chlorpyrifos [organophosphate]	Methyl parathion, methamidophos [organophosphate]
Use of persistent organic pollutant pesticides (eg DDT)	Heavily used in anti malaria campaign (1945-75)	Heavily used for cotton cultivation (1955-90)
Effect of mechanization	Manual workload reduced remarkably after introduction of machines (specially mini-tractor) in early 1990s.	35% reduction of work load
Natural contaminants in ground water	Fluoride, high hardness	Arsenic, high hardness

Nephrol Dial Transplant Review Channa Jayasumana et al 2016 (in press)

CINAC definition

CINAC (chronic interstitial nephritis in agricultural communities) is a CKD affecting mainly young men, occasionally women, its etiology is not linked to diabetes, hypertension, glomerulopathies or other known causes.

The affected subjects live and work in agricultural communities located in CINAC endemic areas with particular socio-economic-occupational determinants such as tropical climates with poverty, exposed to toxic agrochemicals through work or by ingestion of contaminated food and water or by inhalation.

Proteinuria is absent or low, kidneys are small with irregular contours in CKD stages 3-4 with tubulo-interstitial lesions and glomerulosclerosis at renal biopsy. Nephrol Dialysis Tranpl. 2016 in press

CURRENT PROGRAM

Screening at early stages of the disease : tubular , glomerular

Renal biopsies of CKD2 patients with CINAC

Proteomics of parafin embeded renal biopsies of CKD2-3 CINAC patients

Liquid chromatography coupled to high-resolution mass spectrometry (LC-QTOFMS) of water , urine …. Samples in endemic and non endemic areas

Universiteit Antwerpen

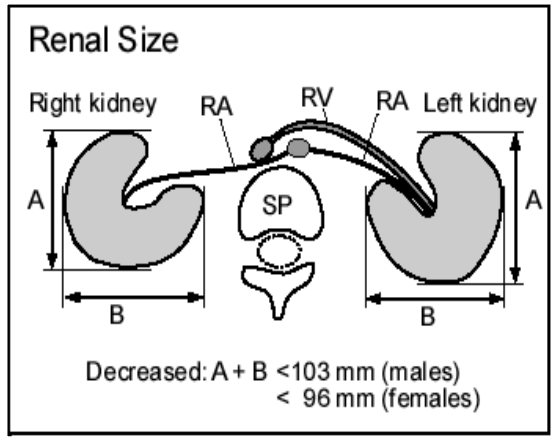
Water samples collected from locations situated in the affected regions will be subjected **to a non-targeted screening by using liquid chromatography coupled to high-resolution mass spectrometry (LC-QTOFMS),** a very powerful technique used for identification purposes. This technique allows the measurement of accurate masses (with three decimals) of ions from compounds present in the samples. The obtained full-scan mass spectrum is then used for the identification of the compounds through search in libraries or chemical databases. Further confirmation will be done by performing MS/MS experiments (formation of fragment ions) and using data mining specific software.

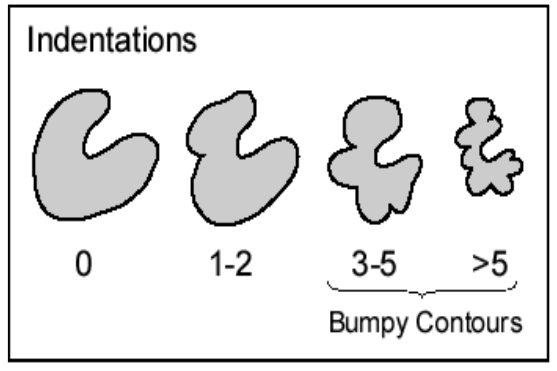
INTERNATIONAL SOCIETY OF NEPHROLOGY

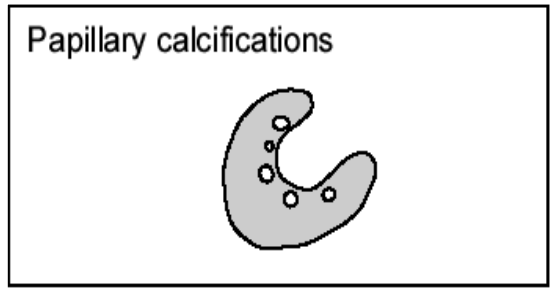
ISN

1960

Analgesic Nephropathy: Measurement of Diagnostic criteria





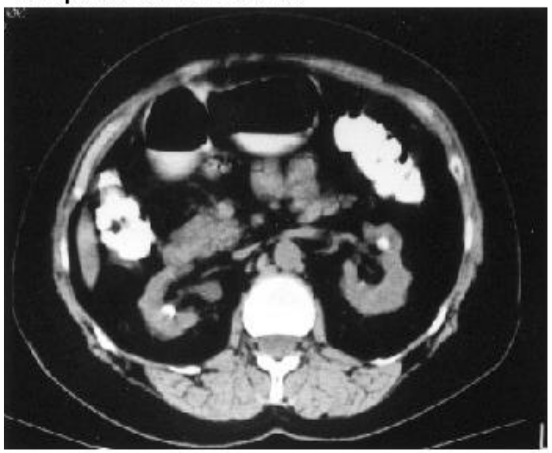


CT scans without contrast material

End-stage renal failure

Belgian female, age 59y, ESRF.
Abuse: 8 y mixture of pyrazolone derivatives
26 y mixture of aspirin + paracetamol

Incipient renal failure



Belgian female, age 62 y, Scr 1.8 mg/dl.
Abuse: 20 y mixture of pyrazolone derivatives

Normal renal function

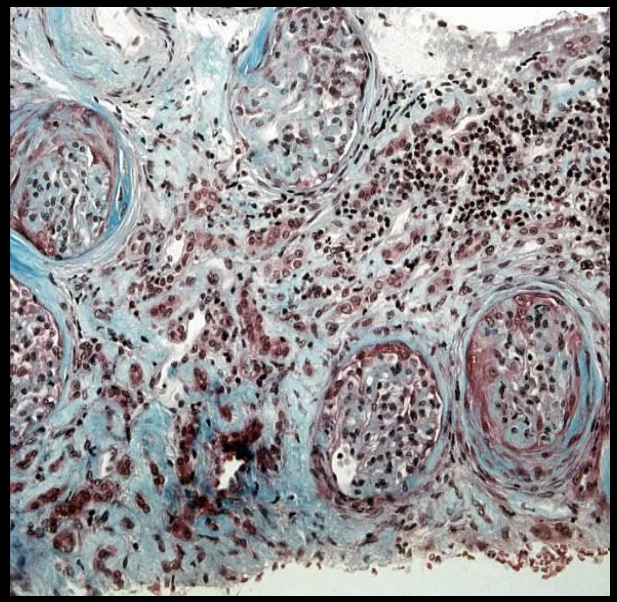
Belgian male, age 66 y, normal renal function.
Abuse: 24 y mixture of aspirin + phenacetin
20 y mixture of pyrazolone derivatives

De Broe ME & Elseviers MM: New Engl J Med 338: 446-452, 1998

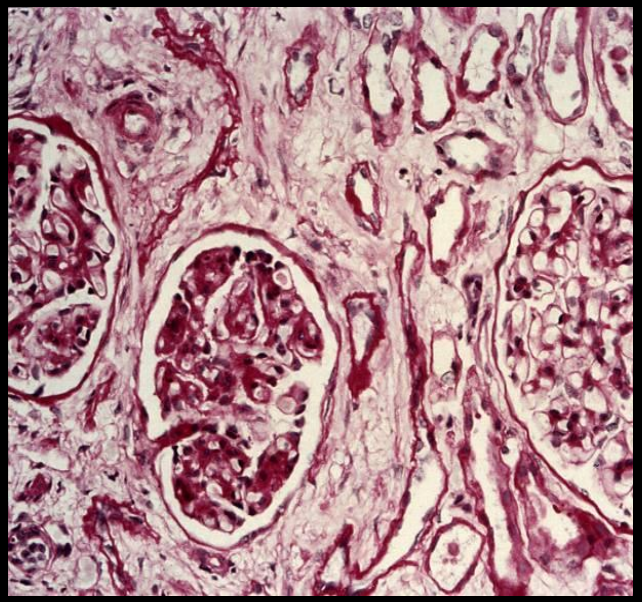
Chinese herbs nephropathy

Kidney biopsy showing tubular atrophy, widening of the interstitium, important cellular infiltration, fibrosis, glomeruli surrounded by a fibrotic ring. Lesions are mainly located in the cortex.

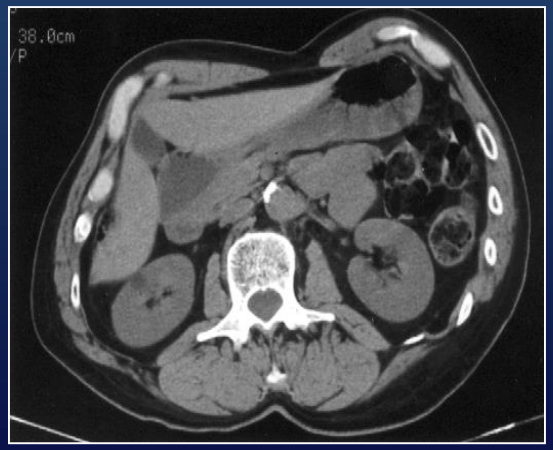
Masson staining



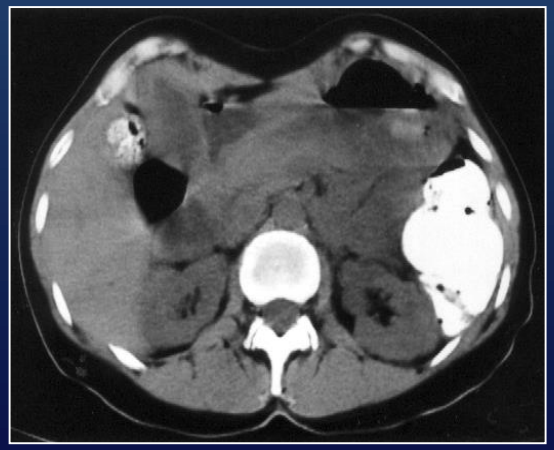
PAS staining



CT scan: Control patient: normal renal function



Chinese herbs nephropathy: irregular contours, no calcifications



Z.L. – female - 43 years
1998: serum creatinine 1.4 mg/dl, polyuria, proteinuria 250 mg/24h
2000: end-stage renal failure, renal transplantation, bilateral uretheronephrectomy